IDENTIFICATION OF GENETIC COMPONENTS FOR RESISTANCE TO AFLATOXIN PRODUCTION IN PRE-HARVEST MAIZE

A Dissertation

by

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ABSTRACT

Aflatoxins, produced by the fungus *Aspergillus flavus*, often contaminate preharvest maize (*Zea mays* L.) grain under heat and drought stresses, and pose serious health hazards to humans and livestock.

Since 2003, a multi-environmental trial of public breeding maize (*Zea mays* L.) hybrids across multiple programs in the southeastern United States has evaluated accumulation of aflatoxin following inoculation with the fungus, *A. flavus*. The Southeast Regional Aflatoxin Trial (SERAT) was formed to identify public germplasm with the most consistent resistance to aflatoxin accumulation and agronomic traits in different environments. Yield and agronomic traits were evaluated in 13 locations, aflatoxin in four, from 2006 to 2015. The 295 experimental hybrids that included tropical and subtropical derived germplasm exhibited lower average levels of aflatoxin and lower average yield versus commercial checks. However, the 13 top-performing experimental hybrids identified in SERAT yielded as much or exceeded check averages, and had aflatoxin levels significantly lower than check averages.

A second study was conducted to investigate changes in differential gene expression (DGE) during seed morphogenesis and maturation in the "aflatoxin resistant" inbred line TX772 when challenged by *A. flavus* through two different methods of ear inoculation; non-wounding (silk channel) and wounding (side needle) in reference to a non-inoculated control. Grain maturity had the largest effect on RNA-Seq DGE, however, within each stage of development, similar up-regulation in expression from either inoculation method was

observed. A larger number of fungal reads were observed in side-needle inoculated samples and a correlation of .65 between fungal read percentages and aflatoxin were found. Sixteen genes previously associated with resistance to pathogens were identified among the transcripts differentially expressed (DE) at $p \leq .05$, FDR $\leq .10$, and fold change ≥ 2.0 over all stages. Others not directly associated with resistance but differentially expressed included six zeins, and eight enzymes controlling carbohydrate metabolism. This study confirmed previously implicated candidate genes for resistance and identified new pathways to control *A. flavus* by investigating a unique maize genetic background.

Together these two studies provide new insights into germplasm and genes to further reduce aflatoxin in a field environment.

DEDICATION

To my husband, Walter, for his unlimited love, faith and support all the way in this amazing journey. Also to our daughter, Misty, for her encouragement and on-target advice in negotiating graduate school. And finally, to my mom and dad, for providing the love and training to pursue the most worthwhile things in life.

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This work was supervised by a committee consisting of Professors Seth Murray, Hong-Bin Zhang and Steven Hague of the Department of Soil and Crop Sciences, and Professor Thomas Isakeit of the Department of Plant Pathology and Microbiology.

A large body of the data analyzed for Section 2 was provided by Professor Seth Murray, and the investigators listed herein, who were co-authors with me of a manuscript published in Crop Science. The other co-authors and contributors to the data include: Professor Thomas Isakeit, Dr. Matthew Krakowsky, USDA-ARS Corn Host Plant Resistance Research Unit, MI State, Dr. Baozhu Guo, USDA-ARS Crop Protection and Management Research Unit MI State, Dr. Xinzhi Ni and Dr. Joseph Knoll, USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA, Dr. Wenwei Xu, Department of Soil and Crop Science, Texas A&M AgriLife Research, Lubbock, Texas, Kerry Mayfield, Chromatin Inc., and Javier Betran, Monsanto, Inc. I, Nancy Wahl, analyzed the data and led the writing of the paper with input from those listed previously.

The RNA-Seq analysis depicted in Section 3 was conducted under the advisement of Professor Hong-Bin Zhang, and Dr. Meiping Zhang of the Department of Soil and Crop Science, and Dr. C. Michael Dickens, of the High Performance Research Computing. Dr. Seth Murray designed the experiment and collected the biological samples in the field. Sequencing was done by BGI. I, Nancy Wahl, processed the samples for RNA extraction,

analyzed the data and led the writing of the paper with input from those listed previously.

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NOMENCLATURE

SERAT	Southeast Regional Aflatoxin Trials
CIMMYT	International Maize and Wheat Improvement Center
CML	CIMMYT maize lines
GEM	Germplasm Enhancement of Maize
LAMA	Latin American Accessions
REML	Restricted Maximum Likelihood Estimation
QTL	Quantitative Trait Locus
DGE	Differential Gene Expression
DEGs	Differentially Expressed Genes
PCA	Principal Component Analysis
LOX	Lipoxygenase

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1. INTRODUCTION

Over the past five decades, an ongoing battle has been waged against the sporadic contamination of food crops by aflatoxin. Aflatoxin is a potent immunosuppressive, teratogenic and carcinogenic secondary metabolite produced by the fungus, *Aspergillus flavus*. While the fungus generally lives as a saprophyte in the soil, it can infect a variety of crops including maize, peanuts, sorghum, cotton and other important food commodities, particularly in subtropical and tropical environments. It appears in pre-harvest maize under conditions of abiotic stress, especially prolonged hot dry weather (Hawkins, Windham, and Williams 2008), and may be increased due to damage and spreading by insects (Betran et al. 2003). Susceptibility may also be affected by nutritive factors such as low soil nitrogen levels even with adequate irrigation (Mutiga et al. 2017). *Aspergillus flavus* produces aflatoxins B_1 and B_2, the former being the most toxic, and thresholds for human and dairy cattle consumption have been established at 20 ppb by the U.S. Food and Drug Administration. Due to the high costs of testing and discarding contaminated grain in developed countries, and the lack of adequate regulation in certain other areas of the world, ongoing research is seeking to improve methods of detection, more effectively manage agronomic conditions, identify and develop germplasm more resistant to infection and/or production of the mycotoxin and determine the genes significantly contributing to the resistance, in addition to understanding the biology of the pathogen, and that of host-pathogen interactions. This dissertation has focused two different approaches in two separate studies to further reduce

and better understand aflatoxin contamination in maize. The first study examined improved maize germplasm by evaluating maize hybrids selected for resistance by multiple research programs to the production of aflatoxin through a meta-analysis of multi-environmental trials. The second approach investigated differential expression of genes associated with the introduction of *A. flavus* to maize kernels, as measured by RNA-Seq analysis.

1.1 Selecting Resistant Germplasm

The process of selecting resistant germplasm in maize has faced some significant challenges, especially because inheritance is quantitative, and thus depends not only on environmental conditions which can vary greatly from year to year, but also on genotype by environment interactions. In addition, there are many confounding factors that can appear as resistance, but actually reduce or avoid overall contamination. Effective field inoculations with fungal spores is critically necessary since the incidence of infection is sporadic and different strains produce different levels of aflatoxin (Adhikari, Bandyopadhyay, and Cotty 2016).There are physical characteristics commonly found in the most resistant germplasm, usually of tropical origin, including grain hardness, maintenance of kernel integrity, and extensive husk coverage. However, hybrids with tropical germplasm are often low yielding, at least partly due to later flowering and lodging (Goodman 2005; Mayfield et al. 2012). Another factor that appears to increase resistance is the amount of hybrid vigor displayed in the offspring of parents that may be susceptible per se, but specifically combine to resist the effects of environmental abiotic stress. Such a phenomenon may have occurred in a large association mapping study involving almost 300 maize lines (mostly resistant) crossed a susceptible line,

Va35 (Warburton et al. 2015) where hybrid vigor as determined by crossing with different heterotic groups, played a large role in reducing overall aflatoxin.

Beginning in the late 1970's concerted breeding trials were initiated, first to identify the best sources of resistance to aflatoxin accumulation, and later to consider yield and better agronomic traits as well (Williams et al. 2015; Scott and Zummo 1990; Betran, Isakeit, and Odvody 2002; McMillian, Widstrom, and Wilson 1993; Guo et al. 2011; Williams and Windham 2012). The Southeast Regional Aflatoxin Trial (SERAT) was formed in 2003 among collaborators in Tifton, GA, Starkville, MS, College Station, TX, Lubbock, TX, and in certain years, others including Lewiston and Kinston, NC, Urbana, IL, and other sites in Georgia and Texas. Its ongoing purpose is to identify inbred lines and hybrids that exhibit consistently low aflatoxin contamination upon field inoculation accompanied by competitive yields, and desirable agronomic traits. To ascertain its effectiveness in achieving its goals, a meta-analysis of the SERAT multi-environmental trial data was conducted on the years spanning from 2006 – 2015, and will be presented in the first of two manuscripts contained herein.

1.2 Gene Expression Profiling

Due to the scarcity of large effect QTL for resistance to aflatoxin accumulation, which makes it difficult to apply marker-assisted selection on key alleles, Dhakal (2017) conducted gene expression profiling based upon a library created by suppression subtraction hybridization (SSH) following side-needle inoculation with a fungal solution of *A. flavus* after mid-silk of the susceptible line, B73, and the resistant line, Mp715. The procedure provided for the amplification of up-regulated sequences in the inbred line of interest, in this case Mp715,

which were cloned into specialized vectors, and then verified with reverse northern hybridization to confirm that the unique clones had arisen from the target transcriptome. DEGs from the SSH library were then submitted to Maize GDB Blast for identification and *in-silico mapping* onto the B73 Ref Gen_v2. A locus lookup tool identified physical locations of markers linked with QTLs associated with resistance to aflatoxin accumulation and that had co-localized with the DEGs. qRT-PCR was employed as well to compare gene expression between inoculated kernels and non-inoculated kernels for each line. The largest group of genes DE in Mp715 compared to B73 pertained to cell metabolism, especially in the synthesis and hydrolysis of starch and mobilization of sugars. Others were in the pathway of lignin biosynthesis, which strengthens cell walls against invading pathogens, receptor protein kinases, bZIP transcription factors, elongation factors 1 and 3, and a number of pathogenesis-related (PR) proteins including chitinase. Aflatoxin levels were also tested in two different years, not only in B73 and Mp715, but other lines differing in susceptibility, and as expected, levels were significantly higher in Va35 and B73, and lowest in Mp715 and Mp313E, with other lines falling in the middle.

Five previous studies of DGE in maize kernels inoculated with *A. flavus* will be briefly presented here, only one of which is discussed in Section 3.

The first to be discussed evaluated DGE of four inbred lines exhibiting more resistance to aflatoxin accumulation than the other three tested. RT-PCR tested DE of 94 stress-related genes, under imposed drought stress relative to expression of a reference gene, but no fungal inoculation. There were more up-regulated genes in the resistant group compared to those in the susceptible group, and in contrast, there were more down-regulated

genes in the susceptible group than the resistant one. Genes recognized as being stress or defense related such as two defensins (Jiang et al. 2011), two antioxidant enzymes, signal transduction mediators, a salt-inducible protein kinase, leucine-rich repeat protein kinase, and four stress response proteins such as heat shock protein 21 (hsp21) were all up-regulated in the resistant group .

The second study utilized the Kernel Assay Screening (KSA) in obtaining transcriptional profiles in maize kernels of a resistant line (Eyl25) compared to a susceptible line (Eyl31) following inoculation with the fungus *in vitro* (Luo et al. 2011). They ran four comparisons from microarray experiments of resistant inoculated versus resistant control, and the same for susceptible, and then resistant inoculated with susceptible inoculated, and resistant control with susceptible control, then subtracted out differential expression due to genotype alone. This normalized DE between resistant and susceptible revealed 75 genes expressed in response to inoculation, 23 of which were expressed in both genotypes. Some of the up-regulated ones in the resistant line included HSP70, salt-inducible protein kinase, late embryogenesis abundant protein, wound inductive gene, glutathione-S-transferase, superoxide dismutase, leucine-rich repeat-like protein, PR-4, chitinase, thionin-like protein, cinnamoyl alcohol dehydrogenase and others. There were many more down-regulated genes in the resistant line in this study that included GST 8, ethylene forming enzyme, chitinase III, a number of peroxidases, antifungal thaumitan-like protein, and many others. One of their main conclusions was that resistant germplasm is characterized by a strong (or stronger) constitutive resistance, as supported by more PR genes were expressed in the non-inoculated resistant controls compared to the susceptible controls. Caution must be exercised, however,

in comparing results from in vitro inoculation of imbibed mature kernels separated from the cob to field inoculated kernels.

The third study employed microarray analysis initially to survey candidate genes for DE between highly resistant Mp313E and highly susceptible Va35 following field inoculation with *A. flavus* (Kelley et al. 2012). Following the selection of 50 candidate genes, DGE was determined by RT-PCR between Mp313 and Va35, the expression of each first normalized by expression levels of non-inoculated controls. The most highly expressed gene in Mp313E vs Va35 was a nucleoporin that is believed to regulate transportation of R (resistance) proteins. In addition, some chaperone or heat shock proteins, HSP26, HSP90 and HSP101 were up-regulated along with an ethylene responsive protein belonging to the universal stress protein family. In Va35, a protein associated with the hypersensitive response (programmed cell death), glycine rich RNA binding protein2 was highly up-regulated, as was cinnamoyl CoA reductase, a key enzyme in lignin biosynthesis. Four zein proteins were up-regulated as well, one of them highly significantly, although they have no known role in pathogen resistance.

Another approach was taken in analyzing DGE in maize inbred lines derived from a cross between Mp715 and Va35 (Asters et al. 2014). Of special interest was the identification of DEGs in the RNA transport pathway that included the nucleoporins, and other genes found to be up-regulated in the resistant genotype following field inoculation with *A. flavus* in the previous study (Kelley et al. 2012). RT-PCR was performed, and correlation analysis of expression levels among all pairs in a set of about fifteen genes provided a means of grouping them. A network analysis of groups of these genes based upon their patterns of

expression revealed genes that might be important in regulating host defense responses against the fungus.

Finally profiling the host response to infection by *A. flavus* across four stages of kernel maturity: blister, milk, dough and dent revealed more than 4000 maize genes DE in pooled samples of kernels harvested from the susceptible B73, through microarrays that were validated by RT-PCR (Dolezal et al. 2015). This study is described in more detail in Section 3.

This review would not be complete without at least mentioning one of the latest developments in the resistance to aflatoxin contamination in maize kernels, host-induced gene silencing (HIGS). Before RNA silencing was introduced into a host plant, the technology was shown to be effective in suppressing expression of AflR, the transcription factor required for expression of genes for aflatoxin biosynthesis in transformed *A. flavus* and *A. parasiticus* (McDonald et al. 2005). Six years later, silencingRNA (siRNA) sequences successfully targeted a structural gene aflD, and the regulatory gene aflR in *A. flavus* and *A. parasiticus* protoplasts that reduced aflatoxin B_1 by almost 100% (Abdel-Hadi et al. 2011). The most recent attempt in transformed maize with an RNAi cassette successfully interfered with the transcription of polyketide synthase (aflC), outside of any essential locus in the maize genome (Thakare et al. 2017). The aflC catalyzes the first step in the synthesis of not only aflatoxin B1 and B2, but also G1 and G2 produced by other species of *Aspergillus*. The transformed maize lines (derived from B73), when infected with *A. flavus* produced no aflC transcripts, and no aflatoxins in maize kernels up to the T3 generation.

In consideration of the fact that this technology is relatively new, and needs to be tested in other lines and most importantly, relevant commercial hybrids; it's effectiveness in field settings needs to be demonstrated; finally, it will also require approval by the USDA-APHIS and be shown to be accepted by the general public. The development of aflatoxin resistant lines and hybrids needs to continue, and remains the focus of this dissertation. It is clear that to minimize aflatoxin contamination, multiple approaches will be required.

2. META-ANALYSIS OF THE SOUTHEAST REGIONAL AFLATOXIN TRIALS (SERAT) 2006 - 2015*

2.1 Overview

Aflatoxins pose a serious health hazard to humans and livestock, requiring significant economic cost in identifying and disposing of contaminated grain. Since 2003, a multi-environmental trial of public breeding maize (*Zea mays* L.) hybrids across multiple programs in the southeastern United States has evaluated accumulation of aflatoxin following inoculation with the fungus, *Aspergillus flavus*. The Southeast Regional Aflatoxin Trial (SERAT) was formed to identify public germplasm with the most consistent resistance to aflatoxin accumulation, and to evaluate their essential agronomic traits in different environments. Yield and related agronomic traits were evaluated in 13 locations; aflatoxin in four. From 2006 to 2015, the 295 experimental hybrids, composed of varying percentages of tropical and subtropical germplasm, exhibited lower levels of aflatoxin on average at 323 ppb versus 370 ppb for the commercial checks, while the check average of 10.1 t/ha exceeded the research program average yield by 20%. Repeatability for log-transformed aflatoxin exceeded 0.50 in most years while yield was mostly above 0.75. Testing for Type II stability indicated a positive response of high yielders to better environments. The SERAT program

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enabled the identification of 13 top performing experimental hybrids that have yielded on par with or exceeded check averages, and had aflatoxin levels significantly lower than check averages.

2.2 Introduction

Aflatoxin is a hepatotoxic, carcinogenic and immunosuppressive byproduct of the fungus, *Aspergillus flavus* that is associated with a variety of food commodities such as maize, sorghum, pearl millet, rice, wheat and peanuts among others grown in the tropics, subtropics and the Southern U.S. It continues to cause disease when contaminated grain is consumed in countries that lack regulatory programs and funding to test and control its presence in harvested crops. The level of aflatoxin permitted in grain sold in U.S. interstate commerce is tightly regulated by the U.S. Food and Drug Administration. The current U.S. Food and Drug Administration Guidelines for aflatoxin levels has set a threshold of 20 ppb for feed for dairy cattle, and up to 300 ppb for feedlot cattle, which has resulted in discarded grain each year (FDA regulatory guidance for mycotoxins August 2011), with an estimated impact of $225 million/yr., not including the $20-30 million/yr. for testing (Schmaile III and Munkvold 2009). The most cost-effective way to control aflatoxin is to identify stable sources of genetic resistance which include inbred lines such as Mp313, (Scott and Zummo 1990), Tx772 (Llorente, Betran, et al. 2004), Tx736, Tx739 and Tx740 (Mayfield et al. 2012) Mp718 and Mp719, (Williams and Windham 2012), GT603 (Guo et al. 2011), and TZAR106 (Menkir et al. 2008). Due to its highly quantitative inheritance, however, resistance is not always transferred into high yielding inbred lines. Heritability estimates can be quite variable, often due to the high genotype by environment interaction (GxE) variance.

Aflatoxin accumulation depends on temperature, soil moisture and relative humidity, the genotype of the host and the inoculation method used for screening (Henry et al. 2009; Warburton et al. 2013; Windham et al. 2009; Zummo and Scott 1989). Different inoculation methods (knife, silk channel and side-needle techniques) have all been found to be effective in differentiating among resistant and susceptible hybrids, (Buckley, Williams, and Windham 2006a; Hawkins, Windham, and Williams 2008; Henry et al. 2010; Scott and Zummo 1994; Williams, Windham, and Buckley 2008).

The production of aflatoxin by *A. flavus* is favored by drought stress induced in maize by low rainfall and daily mean temperatures that exceed 25^0C, and a daily maximum above 35^0C (Hawkins, Windham, and Williams 2008). In the US, the states along the Gulf of Mexico and southeast Atlantic coast are most often affected, but there is great variation from one year to the next. In 1977 on the Coastal Plain that includes the Carolinas through Georgia and southern Alabama, the average level of contamination was 97 ppb, but reached more than 600 ppb in some areas (Scully et al. 2009). While drought stress causes the host to be more vulnerable, *A. flavus* grows best with water activity (a_w) between .86 and .96, and being primarily a saprophyte, it lives off plant and animal debris in the soil. It has superior ability to survive and out-compete other organisms under harsh conditions. It overwinters as mycelium or sclerotia that can germinate to produce hyphae and conidia (asexual) spores that are easily dispersed in the soil and air (Bhatnagar, Cleveland, and Payne 2000).

In addition to the abiotic stress conditions, biotic stress due to insect pests that feed on the kernels have been shown to lead to higher aflatoxin contamination; including the southwestern corn borer (*Diatraea grandiosella* Dyar) (Williams et al. 2005) or damage from

stink bugs, *Euschistus servus*, and maize weevils, *Sitophilus zeamais*, (Ni et al. 2011). A diallel study of crosses involving mostly CML tropical and subtropical germplasm conducted in three locations in Texas found that inoculating the ears through the silk channel with a suspension of *A. flavus* spores provided an effective disease challenge to identify differences among the hybrids and in addition, levels of insect infestation and ear rot. In contrast, a study conducted in Alabama did not find any consistent correlation between aflatoxin levels and ear damage due to European corn borer, *Ostrinia nubilalis,* the Southwestern corn borer, *Diatraea grandiosella*, corn earworm, *Helicoverpa zea*, and the fall armyworm, *Spodoptera frugiperda* (Bowen et al. 2014); similarly, no significant differences were found in levels of aflatoxin in *Bt* versus *nonBt* hybrids under natural conditions. While insect damage is an important means by which *A. flavus* can enter the ear and/or kernel, the fungus is capable of growing into the ear via the silks in its absence (Jones et al. 1980). Therefore, insect control alone is not sufficient to control aflatoxin.

A uniform test across different environments and inoculation methods, relevant to perceived *A. flavus* infection mechanisms, is believed to be valuable to identify robust genetic sources of resistance and/or decreased susceptibility. The purpose of a coordinated multi-environmental trial, the Southeast Regional Aflatoxin Trial (SERAT), was to provide breeders a way to identify the most stable lines and hybrids for aflatoxin resistance and characterize their agronomic performance across environments. The contributing programs of the hybrids tested included those at Starkville, MS, Tifton, GA, College Station, TX, Lubbock, TX, Urbana, IL, and occasionally others, although aflatoxin data was only available for the first four locations listed. In addition, other trials using the same hybrids

were conducted in Georgia and North Carolina testing for yield and yield-related traits only. SERAT data have only been previously used on a year-by-year basis internally for breeders to decide on hybrids to advance and determine if their sources of resistance are stable and robust; to date no retrospective analysis or evaluation of the trials has been attempted.

In this meta-analysis,we sought to retrospectively analyze this data to determine: 1. Overall levels of aflatoxin and agronomic performance in terms of yield, plant height, ear height, lodging, and days to flowering in sets of program and commercial check hybrids across different environments. 2. The performances of hybrids using best linear unbiased estimates of yield, yield components and levels of $\log_{10}$(aflatoxin+1). 3. Correlations among different traits within each year of testing. 4. Repeatability and stability of these estimates. This analysis attempted to evaluate the value of the SERAT program and to identify the best performing hybrids, and by extension, the best parental inbred lines for future crosses.

2.3 Materials and Methods

Sources of exotic germplasm

The germplasm used in the SERAT program included diverse hybrids derived from experimental breeding lines, released public program lines, expired plant variety protected lines (ex-PVP's), and commercial testers. The exotic germplasm backgrounds included Tuxpeño, Tuxpan (derived from Tuxpeño), International Maize and Wheat Improvement Center (CIMMYT) lines or (CML), those from the Germplasm Enhancement of Maize (GEM) and other sources such as 100% tropical LAMA lines from Bolivia that had been adapted to temperate climates (Ochs 2005). Tuxpan races are primarily from Mexico, and many released CIMMYT lines include Tuxpeño germplasm (Warburton et al. 2013). GEM

lines have between a 25% to 50% tropical background based on pedigree (GEM ; Nelson et al. 2016). Germplasm from Cuba, known as “Caribbean Flint”, is resistant to maize weevil infestation, and is believed to have originated from the east coast of South America (Hatheway 1957). TZAR106 and five other TZAR lines were developed for resistance to aflatoxin accumulation by the International Institute of Tropical Agriculture (IITA) in collaboration with Southern Regional Research Center of the USDA-ARS (Menkir et al. 2008). Hybrids were made within and submitted by individual breeding programs based on their own criteria, one of the largest of which was seed availability, as producing the required amount of experimental hybrid seed can be challenging in the southern US.

Field design and phenotyping

Two overlapping datasets are reported in this meta-analysis. The first (Dataset 1-yield) included twelve separate locations in which yield and other agronomic traits were measured over ten years, amounting to fifty-four environments, since not every environment was tested in every year. The second (Dataset2-aflatoxin) included the four locations in which aflatoxin was measured over ten years (Starkville, MS; Tifton, GA; College Station, TX; Lubbock, TX), contributing to thirty-one environments.

Each year and across both Dataset1 and Dataset2, a set of four to ten hybrids were contributed by each of four participating breeding programs, and this set of thirty to forty entries were tested at all locations along with several commercial checks to serve as controls. These trials were planted in a randomized complete block design, with two to four replications as the blocking factor. Standard regional agronomic practices with respect to fertilizer and pre-emergent herbicide were followed at each research site. The research plots

were planted at a time when there were high temperatures and low rainfall in order to increase the abiotic stress that promotes the production of aflatoxin; in some locations such as College Station, planting was delayed approximately a month after the optimum planting date. A subset of ears in each plot were inoculated with a suspension of *A. flavus* spores within ten to twenty days following fifty-percent silking (Table 1).

Towards robust screening of resistance, inoculation was performed differently at each site. In College Station, inoculum was prepared from the *A. flavus* isolate NRRL 3357 that had been cultured on potato dextrose agar plates and then applied to sterilized corn kernels for additional growth. The spores were then washed off and purified by repeated sedimentation at 4^0C to achieve a final concentration of 10^7mL just before use. Three ml of inoculum was injected down the silk channel of 10 ears 10-12 days after midsilk. The remaining ears were harvested, weighed and tested for moisture to provide yield per plot together with that from the inoculated ears. In Mississippi, primary ears of the plants in

Table 1. Methods of inoculation

Program	Application	DAS†	Harvest
College Sta., TX	Silk channel	10-12	≥60 DAP‡
Tifton, GA	Knife	20	≥60 DAP
Starkville, MS	Side needle	7	≥60 DAP
Lubbock, TX	Silk channel	10	≥60 DAP

† Days after silking

‡ Days after planting

each plot were inoculated by side-needle technique with a 3.4 ml suspension of 3×10^8 conidia of the *A. flavus* isolate NRRL 3357, 7 days after midsilk (Zummo and Scott 1989). In

Lubbock, 3 ml suspension of 1 x 10^8 conidia of the *A. flavus* isolate NRRL 3357 were injected into the silk channels of primary ears of 12 plants in each plot 10 days after midsilk. In Georgia, the inoculum of 1 x 10^6 concentration of conidial spores was applied by knife to about five kernels through the husk, 20 days after midsilk. In all locations, inoculated ears from each plot were hand-harvested at least 60 days following midsilk, dried, shelled and bulked before weighing and grinding the grain. Fifty gram (or for Tifton 100 g) samples were tested for aflatoxin concentration using the VICAM AflaTest ®per manufacturer's instructions. Because aflatoxin concentration is generally not normally distributed, the levels were adjusted to Log_{10}(aflatoxin+1), as many other authors have reported (Henry et al. 2013; Kang, Lillehoj, and Widstrom 1990; Mayfield et al. 2011).

In addition to yield, plant height and ear height of the average-sized plant in a plot were measured from the ground to the top of the tassel or node of the primary ear, respectively. Also noted were percent lodging of stems and roots, and days to 50% silking and 50% anthesis, although not all traits were recorded in all environments (Tables 2, 3, Appendices 1 and 2).

Statistical methods

Data from all trials were analyzed using the statistical software, JMP®, Version*12 (*SAS Institute Inc., Cary, NC). An all random model in restricted maximum likelihood estimation (REML) was used to accommodate the unbalanced nature of the data, produce variance components and determine the best linear unbiased predictors (BLUPs) as follows:

$$Y_{ijk} = \mu + g_i + e_k + (g \cdot e)_{ik} + \left(\frac{r}{e}\right)_{jk} + \epsilon_{ijk} \quad (1)$$

where μ is the grand mean, g_i is the random effect of hybrid i, e_k is the random effect of environment k, $(g*e)_{ik}$ is the random interaction effect between hybrid i and environment k, $(r/e)_{jk}$ is the random effect of replication j nested in environment k and ε_{ijk} is the random residual effect for hybrid i, in environment k and replication j. This model was used to generate the BLUPs by year in order to test stability of yield and $\log_{10}$(aflatoxin+1), determine genotypic correlations among traits, and rank the program hybrids for yield and level of aflatoxin contamination by year, comparing them with commercial checks.

Table 2. Yield and yield components (dataset 1)

Year	No. of locations	Yield Mg ha^{-1}	Min	Max	Yield program	Yield checks	Program/check averages %	Plant Ht. cm	Ear Ht. cm	Lodging Stem %	Lodging Root %	DTS†
2006	2	9.4±0.2	2.1	15.4	9.1	10.2	90%	199.7	100.6	-	-	
2007	4	10.5±0.1	3.3	16.9	9.8	12.6	78%	248.3	108.8	3.0	1.9	
2008	6	7.9±0.1	0.7	15.5	7.7	9.8	78%	245.5	98.7	3.9	1.7	
2009	8	8.5±0.1	1.2	16.3	8.3	10.4	80%	269.1	114.7	2.0	0.9	
2010	6	7.7±0.1	0.5	17.9	7.5	9.2	82%	262.8	110.2	2.6	8.3	
2011	6	6.8±0.2	0.2	17.6	6.6	7.7	86%	234.1	91.8	0.1	5.3	60.06
2012	5	8.2±0.1	2.4	17.7	7.7	10.2	76%	262.1	103.6	4.3	20.5	63.81
2013	5	8.1±0.1	1.2	15	7.9	9.3	85%	250.9	102.9	2.6	2.4	66.01
2014	6	9.4±0.1	1	19.4	9.1	10.6	86%	233.7	91.3	1.1	0.2	67.02
2015	6	8.8±0.1	1.5	19.6	8.3	10.7	78%	267.3	107.4	2.9	10.4	59.05
Average		8.5±0.0	0.2	19.6	8.1	10.2	80%	250.7	103.2	3.5	3.6	63.19

† DTS, days to silking

Table 3. Aflatoxin levels of all maize hybrids by year (dataset 2)

Year	No. of locations	Aflatoxin ng g^{-1}	Log_{10}(afl+1)	GM PPB †	L 95 CI	U 95 CI	Program GM PPB †	Checks GM PPB †	Program/check averages %
2006	2	403.4	2.32	209.8	179.7	245.0	190.5	291.6	65%
2007	3	545.3	2.42	259.8	220.2	306.6	242.2	326.0	74%
2008	3	597.0	2.43	267.4	232.4	307.8	247.8	469.9	53%
2009	3	217.5	2.01	101.1	83.9	121.8	95.0	331.0	29%
2010	2	248.4	2.09	121.0	99.1	147.8	113.1	251.7	45%
2011	4	422.7	2.40	249.6	222.2	280.4	262.4	187.2	140%
2012	4	338.1	2.08	119.7	101.9	140.5	118.0	127.1	93%
2013	3	192.5	2.02	102.9	90.5	117.0	99.0	125.7	79%
2014	4	192.1	1.93	83.6	74.1	94.3	74.0	124.7	59%
2015	3	178.6	1.96	91.1	79.8	104.1	76.9	179.2	59%
Average		323.3	2.1	139.0	132.4	146.0	130.4	187.2	70%

† Geometric mean of back-transformed from log(afl+1)

A second mixed model in REML was used to test the magnitude of the random effects of the program hybrids while keeping the check hybrids fixed, and the environment and all interactions random as follows, producing a table of variance components and one of fixed effects:

$$Y_{ijk} = \mu + p_i + c_i + e_k + (p \cdot e)_{ik} + (c \cdot e)_{ik} + \left(\frac{r}{e}\right)_{jk} + \epsilon_{ijk} \quad (2)$$

where μ is the grand mean, p_i is the random effect of program hybrid i, c_i is the fixed effect of check hybrid i, e_k is the random effect of environment k, $(p*e)_{ik}$ is the random interaction effect between program hybrid i and environment k, $(c*e)_{ik}$ is the random interaction effect between check hybrid i and environment k, $(r/e)_{jk}$ is the random effect of replication j nested in environment k and $\mathcal{E}_{ijk}$ is the random residual effect for hybrid i, in environment k and replication j.

For these analyses, it was assumed that all test hybrids were unrelated and not randomly chosen, thus repeatability was estimated instead of heritability, on a genotype mean basis. Replications reflect the weighted averages of replications among locations in a given year that ranged from two to four. The variation in the number of locations for testing yield and other traits was considered as well; in most years these included College Station, Tifton, Starkville and Lubbock for yield and aflatoxin, and Kinston and Lewiston, NC for yield. Repeatability (h^2) was calculated as:

$$h^2 = \frac{G}{G + \frac{GxE}{r} + \frac{\epsilon}{r \cdot e}} \quad (3)$$

where G, GxE and Ɛ are the variance components of genotype, genotype by environment interaction and residual error respectively, with r as number of replications and e as number of environments.

A joint-regression analysis proposed by (Finlay and Wilkinson 1963), based upon use of regression by (Yates and Cochran 1938), was applied to determine stability of yield BLUPs in each of the genotypes, i.e. level of sensitivity in response to different environments relative to the average genotype. In their model, the individual performance of a genotype is regressed against the average of all the genotypes in a given environment, in order to obtain one of two types of stability. Both Types I and II are based upon the slope (b) itself: Type I, b = 0 indicates no difference in level of performance in poor and favorable environments alike, and Type II, b = 1, the entry responds in a similar manner to the average of all entries, responding favorably to more favorable environments. Since most program hybrids were only tested in one year, separate analyses were run for each year. The minimum number of environments required for the regression analyses was set to five, thus certain hybrids that were not tested in enough locations in a given year were not included in this analysis. A third type of stability, Type III, measures the mean square deviation from the regression (mean square error) (Joppa, Lebsock, and Busch 1971).

2.4 Results and Discussion

Ranges and averages for yield and agronomic traits in dataset 1

The average yield over all environments and entries was 8.5 t/ha with a wide range of 0.2 to 19.6 t/ha as shown in Table 2 (Dataset 1). The lowest yields were observed in Starkville, MS and Lubbock, TX in 2011, when the southern U.S. experienced prolonged high temperatures and record low precipitation during the summer months. Program entries averaged 8.1 t/ha with a range of 0.2 to 17.6 t/ha, and overall, the check entry average exceeded this by 20% at 10.1 t/ha. Tifton, GA had the highest average yield across years, and the North Carolina sites had the lowest, most likely due to being the most geographically north, however, a wide diversity of yields was observed between years **(**Appendix 1). In comparison, a diallel analysis of hybrids of certain GEM lines crossed to aflatoxin resistant or aflatoxin susceptible lines in the Mississippi program conducted in MS 2010, 2011 and CS 2011 had average yields of 5.6, 4.3, and 8.2 t/ha respectively (Henry et al. 2014), while SERAT yields in the same environments with similar hybrids were 6.5, 3.7 and 8.5 t/ha respectively. Given the inclusion of exotic germplasm for aflatoxin resistance, it was unsurprising that the temperate commercial check average yields exceeded average yields of the program entries. Tropical lines are good sources of pest or disease resistance, but tend to have delayed flowering and maturity, photoperiod sensitivity, more lodging and excessive ear height among other undesirable agronomic traits that have discouraged most breeders from using them in breeding (Goodman 1999; Mayfield et al. 2012; Nelson and Goodman 2008). Both program entries and check entries averaged 250 cm for plant height, although checks were 8 cm lower than the average program ear height of 104 cm. The program entries exhibited twice as much

stalk lodging (2.8%) compared to the checks, but both groups experienced similar root lodging (4.5%).

Ranges and averages for aflatoxin levels in dataset 2

The average level of aflatoxin measured in inoculated ears over all environments for all entries was 323 ± 10 ppb, with 313 ± 12 ppb for program hybrids at 84% of the 370 ±23 ppb for checks (Dataset 2 - Table 3) and (Appendix 2). Applying logarithmic transformation, $\log_{10}$(aflatoxin + 1), to the raw data increased the percentage of genetic variance and reduced the residual error, and so was retained in subsequent analyses.

Following log transformation, the geometric mean of back-transformed values was 139 ppb for all entries over all environments. Confidence intervals for geometric means were calculated on the transformed scale and then transformed back, as the standard deviation cannot be transformed back to the original scale (Bland and Altman 1996). This was the only trait with an estimate significantly different between program and check hybrids with 95% CI for program hybrids of 123-138 ppb below that of the check hybrids at 169-207 ppb. Each location in some years experienced unusually high levels of contamination, such as in College Station, TX in 2011 when there was a prolonged season of high temperatures and drought, and unlike most years, program hybrid levels exceeded check levels of aflatoxin. Levels in Lubbock, TX were also high in 2011 relative to that of other years, and as in College Station, the average level for program hybrids exceeded the check average. Since the methods of inoculation (along with agronomic management and environmental conditions) varied by location (Table 1) the levels are not directly comparable; however, there was consistency of hybrids between locations, which is important for the practical resistance

desired by growers.

Analysis of variance components

Most hybrids, except for certain commercial checks, were only evaluated as a set in one year in multiple locations, each with varying number of replications. Variance component effects due to hybrid, therefore, were summarized by year for yield, aflatoxin levels, plant height, ear height, lodging, days to silking, days to anthesis and over multiple locations. In the all-random model that included data across years and environments, Eq. (1), the variation in yield due to genotype was 18.5% of the total variation (significant at $p < .0001$) which exceeded the residual variation, (Table 4a) by 2%. As expected, the environmental difference in yield dwarfed the genotype effect, but GxE was only half of the genotypic variation. There was no one location that contributed to these effects, and high genetic variance in yield and other agronomic traits due to environment and genotype by environment interactions is common especially under drought conditions in maize (Farfan et al. 2015; Zaidi et al. 2004). Since the trials were conducted at a later than optimal planting date, drought and heat stress occurring during flowering and grain fill stages likely had a significant impact on many genotypes, even those of tropical origin. This can be exemplified by the results of another trial conducted in College Station on maize in 2011, under extreme water stress (Farfan et al. 2013); in 2011 the environment component reached 70% compared to 2012 at 42% using the same hybrids. In regard to $\log_{10}$(aflatoxin) in Dataset2, genotype and environment contributed nearly equally to the variance in $\log_{10}$(aflatoxin + 1) at 22 and 25% respectively, over all years, (Table 4b.). But the residual variance at 39% was much higher than that for yield, as expected given the complexity of the mechanisms of response to infection by *A.*

flavus and mycotoxin production. The large genotypic component in this dataset suggests that stable genetic mechanisms could be identified across environments and inoculation techniques for aflatoxin resistance. Although there was a large GxE effect for many of these traits, we were looking for stable yield and aflatoxin resistance (genetic variance) so we continued to combine all environments in further analysis to simplify presentation.

Table 4. Summary of random effects under model 1

	Variance components					
Random Effect	**a. Yield**		**Percent Yld**	**b. Log10(aflatoxin + 1)**		**Percent Aflatoxin**
Pedigree	1.63 ± .15	***	18.51	0.09 ± .01	***	21.85
Environment	4.84 ± .96	***	54.88	0.10 ± .03	***	25.17
Environment*Pedigree	0.81 ± .05	***	9.13	0.0 5± .01	***	13.26
Replication[Environment]	0.1 ± .02	***	1.13	0.00 ± .00		0.41
Residual	1.44 ± .04		16.34	0.1 6± .00		39.31
Total	8.82			0.40		

* Significant at the .05 level
** Significant at the .01 level
*** Significant at the .001 level

Table 5. Summary of random and fixed effects under model 2

	Variance components			
Random Effect	**a. Yield**	**Percent Yld**	**b. Log10(aflatoxin + 1)**	**Percent Aflatoxin**
Program hybrid	1.44 ± .15	15.99	0.09± .01	21.91
Environment	4.9 ± 1.02	54.32	0.08± .02	20.95
Program*Environment	0.74 ± .05	8.25	0.05± .01	12.82
Check*Environment	0.39 ± .09	4.30	0.02± .01	5.10
Replication[Environment]	0.1 ± .02	1.10	0.00± .00	0.40
Residual	1.45 ± .04	16.04	0.15± .00	38.84
Total	9.02		0.40	
c. Fixed effects of checks only		***F Ratio***	***P > F***	
Yield		4.04	<.0001	
Log(Afl)		3.52	<.0001	

In the second model, Eq. (2), program hybrid entries were coded as random, and commercial checks as fixed. Eliminating the commercial checks, the genotypic variance component effects on yield decreased to 16%, and the fixed effect of checks was significant at $p < .0001$. This mixed model was applied to $\log_{10}$(aflatoxin + 1) as well. Under these constraints, the genotypic variance component effects of program hybrids were 22%, and checks were significant at $p < .0001$ (Table 5a, b and c). Comparing the results of these two models indicated that most of the genotypic variation came from the program hybrids, which exhibited a wide range in agronomic traits, but also greatly outnumbered the total number of checks tested.

Repeatability for yield, aflatoxin and other agronomic traits

SERAT entries were assumed to be unrelated, so repeatability Eq. (3) was calculated instead of heritability based on the all random model Eq. (1). Repeatability for yield by year from 2007 through 2015 ranged from .73 to .89 (Table 6.), demonstrating good consistency in year to year variation. In 2006, the repeatability was zero as the hybrids responded very differently in the two locations of testing for which data were available. The values for yield were comparable or slightly exceeded those reported for two other hybrid trials conducted in College Station and Corpus Christi, one evaluated 48 testcrosses of quality protein maize adapted to southern U.S. temperate climates (Betrán et al. 2006), and the other evaluated testcrosses between a Texas stiff stalk line and about 350 diverse maize inbred lines representing a mix of tropical, sub-tropical and temperate germplasm (Farfan et al. 2015). The repeatability of plant height was generally higher than that of yield from Dataset 1, likely due to fewer diverse testing locations. Stem lodging varied greatly from year to year while

days to silking exhibited a high repeatability of mostly 0.9 and above, which was observed in the 350 testcross study, but exceeded the range in 48 testcross study. Although a smaller set of environments was used (Dataset 2), the aflatoxin repeatability values ranged more widely than the agronomic traits (Table 7.). As expected, the repeatability values for aflatoxin also had a wider range than that for $\log_{10}$(aflatoxin + 1), of 0 to .90 and .52 to .84 respectively. The values for $\log_{10}$(aflatoxin + 1) especially were within the range observed for the previously referenced studies. The lowest repeatability for $\log_{10}$(aflatoxin + 1) were demonstrated that despite diverse environments and inoculation methods, the different programs could reasonably expect to separate broadly resistant hybrids (program or commercial checks) from susceptible germplasm. However, as different hybrids showed different repeatability, without multiple locations and stability analysis, it would be difficult to ensure that a hybrid had broad resistance in those years.

Table 6. Repeatability on dataset 1

	No.of replications	No. of locations	Yield	No. of locations	Plant height	No. of locations	Stem lodging
Year			**h^2**		**h^2**		**h^2**
2006	3.0	2	0.00	1	0.59		
2007	3.0	4	0.89	3	0.92	3	0.88
2008	2.5	6	0.89	5	0.79	4	0.30
2009	2.5	9	0.73	6	0.89	6	0.50
2010	2.5	6	0.82	4	0.95	4	0.52
2011	2.5	6	0.80	5	0.75	4	0.00
2012	2.5	5	0.81	4	0.94	3	0.09
2013	2.5	5	0.80	4	0.78	3	0.40
2014	2.5	6	0.76	3	0.88	2	0.26
2015	2.5	6	0.89	4	0.92	3	0.92

Table 7. Repeatability on dataset 2

	No.of replications	No. of locations	Aflatoxin	Log10(aflatoxin+1)	No. of locations	DTS
Year			**h^2**	**h^2**		**h^2**
2006	3	2	0.00	0.52	2	0.96
2007	3	3	0.34	0.62		
2008	3	3	0.37	0.73		
2009	2.5	3	0.67	0.72		
2010	3	2	0.00	0.09		
2011	3	4	0.42	0.68	3	0.85
2012	2.5	4	0.12	0.77	3	0.90
2013	3	3	0.90	0.84	3	0.97
2014	3	4	0.43	0.70	4	0.97
2015	3	3	0.80	0.66	3	0.97

Correlations

Correlations were calculated phenotypically with raw data and genotypically using BLUP estimates, Eq. (1) within each year (Appendix 3). Phenotypic correlations between yield and plant height were generally positive, but often not significant, at least partly due to yield being determined by a pooled grain weight for each plot, while height was taken on one average plant per plot (Appendix 1). No consistent relationship between ear height and yield was found, but a strong positive relationship between plant and ear heights was identified as expected. More than half of the correlations between plant height and days to silking (DTS) were positive and highly significant phenotypically and genotypically. Small and mostly negative correlations were observed between yield and days to silking. This is commonly observed in the southern U.S., especially for late planted material exposed to additional heat later in the season. High temperatures reduce growth through shortening developmental phases, which reduces grain filling (Lee and Tollenaar 2007). Two important components of

maize yield are the number of grains and their average weight (Gambín, Borrás, and Otegui 2006; Ordonez et al. 2015), the first of which is set within a 30-day period around silking, and the second being realized during the grain-filling period. During high temperatures, female tissues are also adversely affected as is pollen sterility.

Correlations between $\log_{10}$(aflatoxin + 1) and DTS were mostly negative, especially in Georgia and Mississippi (Appendix 3). A negative relationship has been observed in other studies as well; in the 48 testcross study (r = -.35 phenotypic and -.76 genotypic) (Betrán et al. 2006), and also in one conducted on 25 commercial field and food hybrids evaluated in College Station and Weslaco, TX (r =- .73) (Betran and Isakeit 2004). In the latter study, the authors attributed this observation to a confounding factor of more temperate germplasm adapted to the Midwest flowers earlier in Texas but is also more susceptible to aflatoxin production, compared to those more adapted to southern environments; a challenging hypothesis to test. In the former study of 48 test crosses, the relationship was attributed to germplasm differing in endosperm texture, kernel integrity and susceptibility to aflatoxin. Flinty genotypes have been shown to accumulate less aflatoxin contamination, but their origins are mixed from across the Northern U.S. as well as lowland Central and South America. A third study that tested tissue-specific components of the response to inoculation with *A. flavus* in maize inbred lines, attributed the negative correlation of flowering time with aflatoxin accumulation to either environmental differences (since inoculation was conducted somewhat later in the season on later maturing lines) or physiological differences in later maturing varieties (Mideros et al. 2012). In contrast to the findings in the three aforementioned studies, (Mayfield et al. 2011) reported a positive genotypic correlation

between flowering time and aflatoxin possibly indicating a late season environment favorable to aflatoxin accumulation in the bi-parental recombinant inbred lines tested. They further noted that certain quantitative trait loci for aflatoxin, silking and yield co-localize on chromosome 9, and believed that the silking QTL is the same as that detected in other studies (Buckler et al. 2009; Chardon et al. 2004). The divergence of findings in the direction of correlation between silking and aflatoxin accumulation suggests that it may be a germplasm dependent and/or an environmentally dependent relationship. Across diverse environments and diverse germplasm studied here the relationship clearly appears negative.

In the present study, there was no consistent relationship between yield and aflatoxin levels on a genotypic or phenotypic basis by year (Appendix 3) or BLUPs for each genotype using all years combined. However, in 2006, a significant negative relationship between the two was observed, whereas in 2009 and 2014 there was a significant positive relationship. Also, certain entries tested in multiple years tended to exhibit exceptionally low aflatoxin levels and low yields, particularly hybrids from the Mississippi program. A few had low aflatoxin levels and relatively high yield. This is a bias of the SERAT test however, where better performing entries (including commercial checks) are more likely to be replicated in subsequent years than poor performing entries. It should be noted that no correlations between yield and $\log_{10}$(aflatoxin + 1) is displayed for Tifton, GA because they were tested in separate fields, and often different farms each year. Both the 48 testcross study (Betrán et al. 2006) with limited irrigation and the 25 commercial hybrids study with irrigation (Betran and Isakeit 2004) showed significant negative correlations between yield and $\log_{10}$(aflatoxin), although the former also tested the inbreds per se, and noted significant

positive correlations. It seems likely that in many environments, robust genotypes experience less stress and thus accumulate less aflatoxin and this confounds detection of other aflatoxin resistance mechanisms.

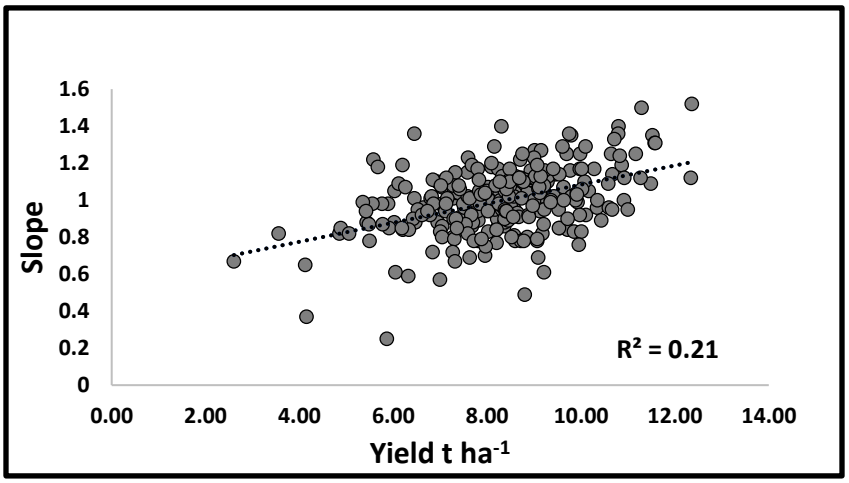

Figure 1. Regression coefficients related to yield stability plotted against yield best linear unbiased predictors of hybrids in different locations from 2008 to 2015.

Stability Analysis

In the current study, there was a highly significant positive relationship between yield and slope, ($r = .45$, $p<.0001$) (Figure 1), with most of the top yielding hybrids having a slope between 1.0 and 1.4. Thus higher yielding entries tended to be less stable as they performed better in more favorable environments, and less well in unfavorable environments. Significant correlations between yield and slope were especially evident in the years from 2010 to 2015 (Table 8.). This suggests that it is unrealistic to expect high-performing genotypes to produce outstanding yields under all conditions, but to utilize the available

moisture and nutrients in a more efficient way than other relevant genotypes, and exhibit greater resistance to the detrimental effects of biotic and abiotic stresses. In general, most breeders no longer consider Type I desirable, where the genotype always shows a constant yield regardless of environment, otherwise known as the 'biological concept' of stability (Becker 1981) versus the 'agronomic concept of stability' which favors the capacity for improvement in response to the environment. The ideal variety, according to (Finlay and Wilkinson 1963), is one with 'maximum yield potential in the most favorable environment', which is in agreement with the conclusions of (Betran et al. 2003; Perkins and Jinks 1968); most of the best lines in SERAT demonstrate an agronomic concept of stability. Values for stability along with average yield for each line tested in each year are provided in Appendix 4, although some lines were not included in enough environments for stability to be calculated**.** Stability could not be measured for aflatoxin levels in hybrids since there were at most four environments each year. The market for hybrid seed is more limited in South than in the Midwest, therefore it is even more important to identify hybrids that are broadly outstanding under all of the diverse environmental and management conditions that the southern US corn region experiences.

Table 8. Pearson's correlation of yield and slope (β) in Type II stability

Year	r	p
2008	0.28	0.101
2009	-0.01	0.939
2010	0.53	0.002
2011	0.51	0.011
2012	0.71	<.0001
2013	0.66	<.0001
2014	0.50	0.001
2015	0.70	<.0001

Type III stability was measured by the mean square error (MSE) for each regression. (Joppa, Lebsock, and Busch 1971) noted that a high MSE was indicative of susceptibility of certain genotypes of wheat to disease. Thus the lack of Type III stability or high MSE can be useful in calling attention to the relative lack of adaptability of a particular variety to different environments. However, in this study no significant correlations between MSE and yield or slope were found, nor were higher MSE values associated with higher levels of aflatoxin.

Stabilities along with BLUPs for yield and $\log_{10}$(aflatoxin+1) for hybrids related to four commonly used inbred lines chosen from each program are displayed in Appendix 5, to serve as examples for consistency of performance and relationships between yield and stability within a group of hybrids. For the Mp13: lines in 2014 and 2015, there was a fair amount of consistency, with low yields relative to check averages, high stability (all slopes <1), and low aflatoxin levels. Most of the hybrids with GT603 also had low yields relative to the checks, slopes less than 1, and low aflatoxin. More specifically, GP282 x GT603

displayed less stability than most other hybrids, and yielded exceptionally well in the Tifton, GA environment in 2011 and again in 2013, but ranged at much lower levels in all other locations. In 2011, overall aflatoxin levels for GP282 x GT603 were much higher than those in 2013, especially due to the conducive environment in College Station. Two other lines worthy of note are CUBA1TEO30 AND CUBA1TEO21 as both formed hybrids with the same non-stiff stalk (NS) in 2013, and NS1 in 2015. Each line performed very consistently in 2013 and 2015 with respect to yield and aflatoxin, although CUBA1TEO30 produced reasonable yields and average levels of aflatoxin, while CUBA1TEO21 performed poorly in both arenas, and in 2013 had a Type II stability of .37, which indicates a lack of responsiveness to improved environments.

Another type of stability analysis, was used to examine the correlation of values of a trait in a common set of hybrids among different locations. It was evident that hybrid performance in yield and $\log_{10}$(aflatoxin+1) was similar in different locations for each year (Table 9). Over all years for aflatoxin levels, each location was highly correlated with the others, with most differences observed between locations within one specific year. The set of hybrids in 2015 performed in the most consistent or stable manner for yield, with all values for r equal to .90 and above, although overall averages by loHybridcation differed in a typical pattern. The correlations for aflatoxin ranging from .67 to .75 were on the lower end of the range of values from 2011 to 2015.

Exceptional Lines and Hybrids for Yield and aflatoxin

Hybrid performance for aflatoxin resistance and yield was evaluated separately by year, since most hybrids were tested in only one year in multi-locations. Appendix 6 highlights the top

yielding and/or lowest aflatoxin accumulating hybrids by year from the full set of entries listed and ranked in Appendix 7. Through linear contrasts based upon the model represented by Eq. (1), thirteen hybrids were identified as having yields on par with the check average for the year, and significantly lower $\log_{10}$(aflatoxin+1) levels at p = .05. One of them, Tx777 x SS3, achieved this distinction twice in 2013 and 2015.

Yield

While very few hybrids were repeated across years, many of those repeated did perform consistently, reinforcing the value of the SERAT test. B110 x BR-1 had a BLUP yield of 11.55 t/ha in 2007, which was 93% of average check levels; in the following year it was 9.21 t/ha, yet was 99% of the average check level. BR-1 is an inbred line developed by the crossing of tropical germplasm BR52051 and a temperate non-stiff stalk line provided by the USDA GEM project. Inbred lines S2B73, S2B73BC, DK-7, PRA96A, C2A632-1a, B5C2, S1W, CUBA1 as well as BR-1 were all developed by Texas AgriLife Research Lubbock breeding program, and selected for drought and heat tolerance. Two other hybrids that were tested in more than one year and ranked in the top group in both years for yield and low aflatoxin were S2B73 x NC300 and Tx777 X S3 (a coded commercial inbred tester line). The CUBA1 x NS hybrid performed well in 2013 in yield at 94% of the check average. Beyond specific hybrids tested in multiple years, a few named inbreds showed up multiple times in these best hybrids (Table 9). NC300 has 100% tropical origin, but had been adapted to a temperate climate by the North Carolina State University (NCSU) program and is among the higher yielding of its class (Goodman 1999; Hawbaker, Hill, and Goodman 1997). Tx777 is one of the most successful among the LAMA lines of Bolivian origin currently undergoing

the formal release process from Texas A&M University in College Station. CML343, which appeared in two top hybrids in this study, was developed by the International Maize and Wheat Improvement Center (CIMMYT). It was in the top ten percent in yield among 88 tropical-exotic inbreds tested in an extensive multi-environmental trial conducted by NCSU (Nelson and Goodman 2008). The Mississippi line Mp317 also performed well in a number of hybrid combinations in 2010, some not significantly different in yield from the highest performers, and aflatoxin resistance was on par with the average check. Mp317 has been noted for its low levels of *F. verticilliodes* kernel infection and fumonison contamination (Henry et al. 2009; King and Scott 1981). One other line of note for yield is TZAR106 tested by the Mississippi program that formed two hybrids within the higher yielding group, and had aflatoxin levels at 64% and 70% of the average check in 2012.

Table 9. Pearson's correlation of yields and log10(aflatoxin + 1) best linear unbiased predictors across locations.

Log(Afl+1)	Yield	CS	GT	MS	LU	KI	LE
2010	CS		0.76***	0.86***	0.73***		0.79***
	GT			0.89***	0.85***		0.92***
	MS	*0.12*			0.81***		0.91***
	LU						0.86***
2011	CS		0.78***	0.85***	0.64***	0.85***	0.76***
	GT	*0.88****		0.82***	0.82***	0.94***	0.95***
	MS	*0.93****	*0.86****		0.74***	0.86***	0.78***
	LU	*0.74****	*0.76****	*0.68****		0.77***	0.82***
	KI						0.95***
2012	CS		0.74***	0.94***		0.92***	0.94***
	GT	*0.74****		0.78***		0.83***	0.75***
	MS	*0.64****	*0.84****			0.96***	0.96***
	KI						0.93***
2013	CS		0.82***	0.74***		0.67***	0.67***
	GT	*0.81****		0.86***		0.84***	0.77***
	MS	*0.80****	*0.86****			0.75***	0.65***
	KI						0.91***
2014	CS		0.64***	0.86***	0.82***		
	GT	*0.79****		0.67***	0.44***		
	MS	*0.71****	*0.70****		0.67***		
	LU	*0.81****	*0.75****	*0.68****			
2015	CS		0.93***	0.94***		0.93***	0.90***
	GT	*0.67****		0.93***		0.90***	0.93***
	MS	*0.75****	*0.72****			0.95***	0.95***
	KI						0.95***

*** Significant at the .001 level

Aflatoxin

In total across locations, 95 hybrids had $\log_{10}$(aflatoxin + 1) levels that were 90% or less than the average of the checks for the years 2006 – 2015, and 25 hybrids had log10(aflatoxin + 1) that were 75% or less than the average of the checks (Table 10). The most common inbreds noted among the 95 low-aflatoxin hybrids include Mp313E, Mp494, Mp717, Mp719, Mp13 series of inbreds, GT601, GT603 and Tx777. Mp313E (Scott and Zummo 1990) stood out as being a parent in nine of the top 25 hybrids and had the only hybrid that reduced aflatoxin by more than 50% of checks (49%, Mp 313E x Mp 719 in 2013). Overall, 18 of the top 25 lines were from the Mississippi program, suggesting useful and broadly-adapted selections for resistance; unfortunately, the yield rank was low for most of the hybrids (with one exception in 2013: Mp313E x NC322). Mp313E was derived from Tuxpan, and was released primarily as a source of resistance to kernel infection by *A. flavus* (Scott and Zummo 1990). A diallel analysis on aflatoxin accumulation in Mississippi found this line, along with Mp494, Mp717 and Mp715 (from which Mp719 is derived) exhibited significant negative (reduced aflatoxin) GCA effects (Williams et al. 2008). TZAR106 (Menkir et al. 2008) stood out as being a parent in two of the top 11 lines for aflatoxin reduction and more importantly on two susceptible temperate testers of each major heterotic group (LH51, LH132); furthermore, both of these hybrids were at or above 90% of yield of the average checks; unfortunately they were only tested in 2012, but this suggests more extensive testing is warranted.

Table 10. Top 25 program hybrids for low aflatoxin levels

Year	Pedigree	Log10(aflatoxin + 1)	% Check average	Yield	% Check average
2008	Mp07:117 x Mp313E	1.87 ±0.27	72%	5.39 ±0.91	58%
2008	Mp04:97 x Mp313E	1.87 ±0.28	72%	5.6 ±0.9	61%
2009	Mp313E x Mp04:97	1.44 ±0.22	65%	6.04 ±0.84	63%
2009	Mp 313E x GT 601	1.57 ±0.25	71%	7.94 ±0.87	82%
2009	Y07-164/LH195	1.63 ±0.22	73%	8.2 ±0.84	85%
2009	B5C2 x NC300	1.67 ±0.22	75%	8.58 ±0.84	89%
2012	TZAR106 X LH51	1.33 ±0.31	64%	8.9 ±1.36	91%
2012	[(Mp494 X GEMN-013) X (Mp717 X GEMS-0074)]	1.34 ±0.25	64%	8.12 ±1.28	83%
2012	TZAR103 X LH51	1.36 ±0.31	65%	8.33 ±1.36	85%
2012	[(Mp317 X mp494) X (Mp717 X Mp313E)]	1.45 ±0.26	70%	7.69 ±1.28	79%
2012	TZAR106 X LH132	1.45 ±0.31	70%	8.8 ±1.36	90%
2012	((Tx741) ; LAMA2002-42-B-B-B-B-B3) X SS3	1.47 ±0.23	71%	8.46 ±1.2	87%
2012	Mp494 X GEMN-0130	1.55 ±0.26	75%	8.07 ±1.28	83%
2013	Mp 313E x Mp 719	1.02 ±0.25	49%	6.94 ±1	74%
2013	Mp 313E x Mp 717	1.35 ±0.25	65%	7.53 ±1	81%
2013	GEMS 0005-2-1B X Hi27bs	1.41 ±0.17	68%	7.59 ±0.75	81%
2013	Mp 313E x NC 322	1.49 ±0.25	72%	8.85 ±1	95%
2014	Mp13:9025 x Mp13:9026	1.31 ±0.23	65%	7.77 ±0.93	74%
2014	GEMS-0028-2-1 x GT603	1.45 ±0.23	72%	8.53 ±0.93	81%
2014	Mp13:9025 x Mp13:9026	1.31 ±0.23	65%	7.77 ±0.93	74%
2014	GEMS-0028-2-1 x GT603	1.45 ±0.23	72%	8.53 ±0.93	81%
2014	Mp13:9031 x Mp13:9032	1.45 ±0.23	72%	8.69 ±0.93	82%
2015	Mp13:9021 x Mp13:9022	1.56 ±0.17	72%	5.63 ±0.86	53%
2015	Mp13:9037 x Mp13:9038	1.59 ±0.17	74%	6.63 ±0.88	63%
2015	(NC300 x Tx714-B/B104-1/CML343)-2-1-B-B-B-B-B-B-B-B-1-B25/TX777	1.6 ±0.17	74%	9.98 ±0.86	95%

2.5 Conclusion

The SERAT consortium has successfully developed and identified maize hybrids and associated inbred lines that limit accumulation of aflatoxin upon infection by *A. flavus* in

different environments and inoculation methods in the Southern U.S. Furthermore, these results were highly repeatable for reduced aflatoxin accumulation across these conditions. Most of the hybrids tested included tropical or sub-tropical germplasm that has been associated with certain physical and physiological characteristics that serve as a barrier to infection or to the metabolic pathways resulting in production and accumulation of aflatoxin, but are not generally features of higher yielding temperate germplasm. We demonstrated that across diverse germplasm under these diverse conditions there was no direct relationship between yield and aflatoxin, and there appears to be two separate reasons why aflatoxin accumulation in some hybrids is low. First, some well adapted high yielding experimental hybrids and checks accumulate less aflatoxin, likely because they are experiencing less plant stress overall. This may partly explain why in 2011, College Station, experiencing a prolonged period of little precipitation and excessively high temperatures from May through August, observed that check hybrids had lower average aflatoxin levels than the program ones. Also, the maize mapping association panel found that the check hybrids B73 x Va35 had aflatoxin levels not significantly different from the mixed tropical subpopulations, which was attributed mainly to a heterotic response between the two inbreds, as both are quite susceptible per se (Warburton et al. 2013). Second, these results clearly indicate that heritable mechanisms of resistance to aflatoxin contamination are unrelated to adaptation and yield (e.g. Mp313E). In this study, the former case appears to be exemplified in certain LAMA hybrids that have proven to be relatively high yielding and moderately resistant, as well as certain hybrids with CML450, CML343, or NC300. Other germplasm that is not as well adapted, but have demonstrated significant resistance over many environments such as

GT603 and many of the MP lines, provide valuable alleles that could be introgressed into elite and higher-yielding domestic germplasm in the future. A major research effort is underway to validate over twenty genomic regions showing high association with low aflatoxin accumulation in more than one environment, (Warburton, personal communication; (Warburton et al. 2015) based upon an aforementioned large genome-wide association study of 300 inbred lines (Warburton et al. 2013). The results of this study in conjunction with a growing understanding of the genes and proteins involved in aflatoxin resistance should largely mitigate the dilemma of choosing between high yielding maize and aflatoxin resistant maize.

3. DIFFERENTIAL GENE EXPRESSION IN RNA-SEQ EXPERIMENT

3.1 Overview

Aflatoxins, produced by the fungus *Aspergillus flavus*, often contaminate preharvest maize (*Zea mays* L.) grain under heat and drought stresses, posing serious health hazards to humans and livestock, and resulting in significant costs to identify and dispose of contaminated grain. This study was designed to investigate the changes in differential gene expression (DGE) during seed morphogenesis and maturation in the "aflatoxin resistant" Argentinian inbred line Tx772 when challenged by the introduction of *A. flavus* through two different methods of ear inoculation; non-wounding (silk channel, used to select Tx772), wounding (side needle) and a non-inoculated control. Grain maturity had the largest effect on overall RNA-Seq DGE. However, within each stage of development, ranging from blister to dent, similar up-regulation in expression of many maize genes following inoculation with either method was observed; a total of 16 genes previously associated with resistance to pathogens were confirmed among the transcripts differentially expressed (DE) at $p \leq .05$, FDR $\leq .10$, and fold change ≥ 2.0 over all stages. The side needle technique produced a larger effect of infection as evidenced by 6,324 fungal reads versus 518 in silk channel and a higher level of aflatoxin. Correlations between approximately 7,000 fungal reads and the number of maize DE genes for each of the eight treatment groups was 0.57 ($p = .143$), and was 0.65 ($p < .001$) with levels of aflatoxin ranging from 0 to 137 ng g-1. This provided an internal measure of effectiveness of inoculation methods, and confirmed candidate genes in a unique maize genetic background for resistance to *A. flavus*.

3.2 Introduction

Evaluation of differential gene expression (DGE) in maize in response to inoculation with *A. flavus* is challenging given the complexities of the pathogenic response across varying genetic backgrounds, under different environmental conditions and with the timing of infection and harvest. Studies up to the present, based on microarray analysis and/or RT-qPCR, have detected similar patterns of expression for some protein coding genes, such as chitinase, but many other detected genes have been novel to specific experiments. Since RNA-Seq can be applied without a reference genome, new gene sequences and sequence variations in the transcribed regions can now be recognized. In the case of maize, a reference genome is available (Schnable et al. 2009) but given the polymorphism of the maize genome, arising from small and large scale rearrangements (Goettel and Messing 2010; Hirsch et al. 2016; Springer et al. 2009), genes responsible for phenotypes of interest may not be present in the reference genome. TX772, a temperate Argentinian inbred featuring a hard and vitreous endosperm (Llorente, Betrán, et al. 2004; Betran, Isakeit, and Odvody 2002) does not share a common pedigree with most U.S. lines of Reid Yellow Dent, Lancaster or Iodent types (Goodman 2005), and is genetically distant based on existing marker data {Smith et al; 2015). Hybrids of Tx772 with BSSS germplasm (Stiff Stalk Synthetic) have exhibited high yield potential under irrigation. Most significantly for this study, Tx772 has shown good general combining ability to resist aflatoxin accumulation under non-wounding silk-channel inoculation, as evaluated using a diallel with certain tropical and subtropical inbred lines in several southern environments (Betran, Isakeit, and Odvody 2002). In the multi-environment Southeast Regional Aflatoxin Trials (SERAT), the four hybrids using Tx772 as a parent

ranked lower in aflatoxin contamination than 75-80% of the other test hybrids, and ranged from 83-93% of the average check level (Wahl et al. 2017).

To date, few studies have published profiles and changing patterns of differential gene expression in *developing* maize kernels (Liu et al. 2012; Lu et al. 2013; Lee et al. 2002), and only one has reported on changes in expression subsequent to *A. flavus* infection (Dolezal et al. 2015). From these studies, kernel maturity has been shown to be significantly associated with gene expression. Maize kernels go through six stages of development beginning with silking (R1), followed by blister (R2), milk (R3), dough (R4), dent (R5) and physiological maturity (R6) (Ritchie et al. 2008). In addition to the length of time following inoculation as a factor in percentage of kernels colonized and infected (Payne 1992), changes in kernel biochemical composition during kernel development would be expected to influence the degree of infection by the fungus, and possibly the levels of aflatoxin contamination. For example, as early as 10 days after pollination (DAP) at the blister stage, certain alpha zeins and gamma zeins are only just detectable (Woo et al. 2001), and reach significant levels by 15 DAP; in mature maize kernels at 55 – 65 DAP, prolamins or zeins comprise about 50% of the proteins (Liu et al. 2008). Starch reserves are also built up in the endosperm from glucosyl, and fatty acids are stored in lipid bodies (Liu et al. 2008). This growing supply of nutrients can support the growth of the fungus which can begin with silk colonization (Payne 1992), and has been found capable of infecting all tissues of immature kernels at different stages within 96 hours following infection (Dolezal et al. 2013). Dolezal et al. (2015) further investigated this effect in a microarray analysis of transcriptional and physical changes in developing maize kernels infected by *A. flavus*, by inoculating ears of the

inbred maize genotype B73 that is highly susceptible to the fungus and production of aflatoxin (Warburton et al. 2013) at stages R2 – R5, and hand harvesting the ears four days later, followed by immediate RNA extraction. They pooled all samples by treatment to gain a full spectrum of DGE relative to those mock-inoculated. Given the complexity of mechanisms occurring in three tissues: maternal, endosperm and embryo, this approach provided a more complete picture of all the genes likely to respond to the presence of the fungus at different stages in the field environment, although the tissues were not tested separately.

A different approach was taken in profiling gene expression with microarray analysis in *A. flavus* itself while colonizing maize kernels. Kernels were harvested from the blister to dent stages in the field, and then inoculated *in vitro* with fungal conidia (Reese et al. 2011). Among the 190 fungal genes analyzed for patterns of expression, many exhibited differential expression (DE) that appeared to be dependent on the stage of maturity in the kernels.

Inoculation methods

Several methods have been developed and applied over the past forty years to inoculate maize, introducing *A. flavus* conidial spores in a suspension of distilled water to the ears of maize. Under natural conditions inoculation is otherwise governed strongly by the environment and presence and prevalence of fungal spores. Inoculation methods can be broadly grouped as "wounding" and "non-wounding". Wounding methods include the side needle technique which involves inserting a needle under the husks and injecting about three ml. of suspension over the kernels, nicking some kernels in the process (Buckley, Williams, and Windham 2006b), as well as pinbar inoculation and the knife technique (Scott et al.

1991) both of which try to carry spores while creating a wound. One non-wounding technique traditionally used at Texas A&M University requires squirting the inoculum down the silk channel under the husk at the tip of the ear without nicking any kernels (Zummo and Scott 1989), this is how Tx772 was identified as having decreased accumulation of aflatoxin. Another non-wounding method is ground kernel inoculation which increases disease pressure by applying colonized kernels in the furrows to sporulate (Odvody et al. 2000; Farfan et al. 2015); this method is analogous to those used in current atoxigenic biocontrol applications of *A. flavus* (Isakeit et al. 2010; Grubisha and Cotty 2015). Non-wounding methods are likely to be better choices for grower-relevant testing of maize in preharvest aflatoxin susceptible production areas where populations of wounding insects are low and wind or other non-wounding natural inoculation are more important. In contrast, the wounding technique typically can produce higher levels of aflatoxin (Buckley, Williams, and Windham 2006b) and are likely better to discriminate levels of succeptibility.

The primary objective of this study was to identify genes significantly differentially expressed between inoculated and non-inoculated Tx772 kernels at a given stage of maturity (blister, milk, dough or dent), focusing on validating those genes previously reported as having contributed to a pathogenic response. A second objective was to compare the effects of two inoculation methods and a non-inoculated control on gene expression at given stages of maturity, with respect to identity, function and degree of fold change, as well as to determine if one inoculation method consistently resulted in a larger number of significantly differentially expressed genes (DEGs). In conjunction with this assessment, levels of

aflatoxin, and the variation and characterization of fungal transcripts in each sample were also determined for evaluation of inoculation success.

3.3 Results and Discussion

Statistics on RNA-Seq transcript and gene assembly

A total of 313.8 million clean reads were obtained from sequencing 24-RNA-Seq libraries constructed from the 24 samples. The clean reads had a quality of > Q20 for an average of 96.64% of the reads, ranging from 95.95 - 97.35%. Each sample had an average number of clean reads of 13.1 million, varying from 10.6 – 13.7 million. A total of 268,720 transcripts (isoforms) resulting from the transcriptome assembly were associated with 152,574 transcripts at the gene level, with an average contig length of 636 bases. Eighty-five percent of the reads aligned concordantly more than one time, and twelve percent aligned exactly once.

Levels of differential gene expression at different stages

The number of clean reads detected in each sample (Table 11. a and b), averaged about 5.6M, and the number of transcripts or loci identified by *de novo* assembly in Trinity ranged from 57.8K – 77.8K over all samples. The total number of DEGs under silk channel inoculation was 315, and that of side needle was 457 with the breakdown according to development stage displayed in Table 11. (d).

Table 11. Number of reads and transcripts by sample

Sample	Read counts (a)	Number of transcripts (b)	Average number reads/transcript (c)	Maize DEGs‡ per treatment (d)	Number of fungal reads (e)	Total reads per type of inoculation (f)	Aflatoxin ng g-1 (g)
AD_1	5,831,702	62,308	94	70	1,924	1,924	137.7
AK_1	5,795,934	60,048	97	261	14	14	missing
AN_1	5,899,959	59,480	99		0		0
BD_1	5,904,363	64,535	91		305		27.1
BD_2	5,793,513	63,711	91		49		0.0
BD_3	5,887,542	61,770	95	18	167	521	7.8
BK_1†	5,726,260	61,295	93		9		6.1
BK_2	4,706,782	57,752	81		13		0.0
BK_3	5,895,009	59,826	99	3	6	28	3.0
BN_1	5,009,778	62,057	81		5		0
BN_2	5,181,385	58,756	88		12		0
BN_3†	5,718,067	63,218	90		1		0
ED_1	5,881,636	71,732	82		715		8.9
ED_2	5,932,908	68,566	87	16	240	955	0.0
EK_1	5,450,481	67,248	81		187		2.7
EK_2	4,943,068	62,714	79	20	107	294	1.8
EN_1	5,302,995	66,341	80		3		0
EN_2	5,779,269	64,644	89		8		0
FD_1	5,855,546	80,208	73		2,704		21.7
FD_2	5,894,548	75,818	78	353	220	2924	3.7
FK_1	5,849,170	77,779	75		93		10.8
FK_2	6,069,033	76,825	79	31	89	182	3.2
FN_1	6,002,760	74,868	80		28		0
FN_2	5,966,850	77,514	77		30		0

† These samples were eliminated from differential expression analysis

‡ Differentially expressed genes at $p \leq .05$, and FDR $\leq .10$, and Log2(FC) ≥ 1.0

Stages of seed development: A - blister B - milk, E - dough, F - dent

Method of inoculation: D - side needle, K - silk channel

(a) Number of reads reflects number of pairs of reads that overlap a fragment of cDNA.

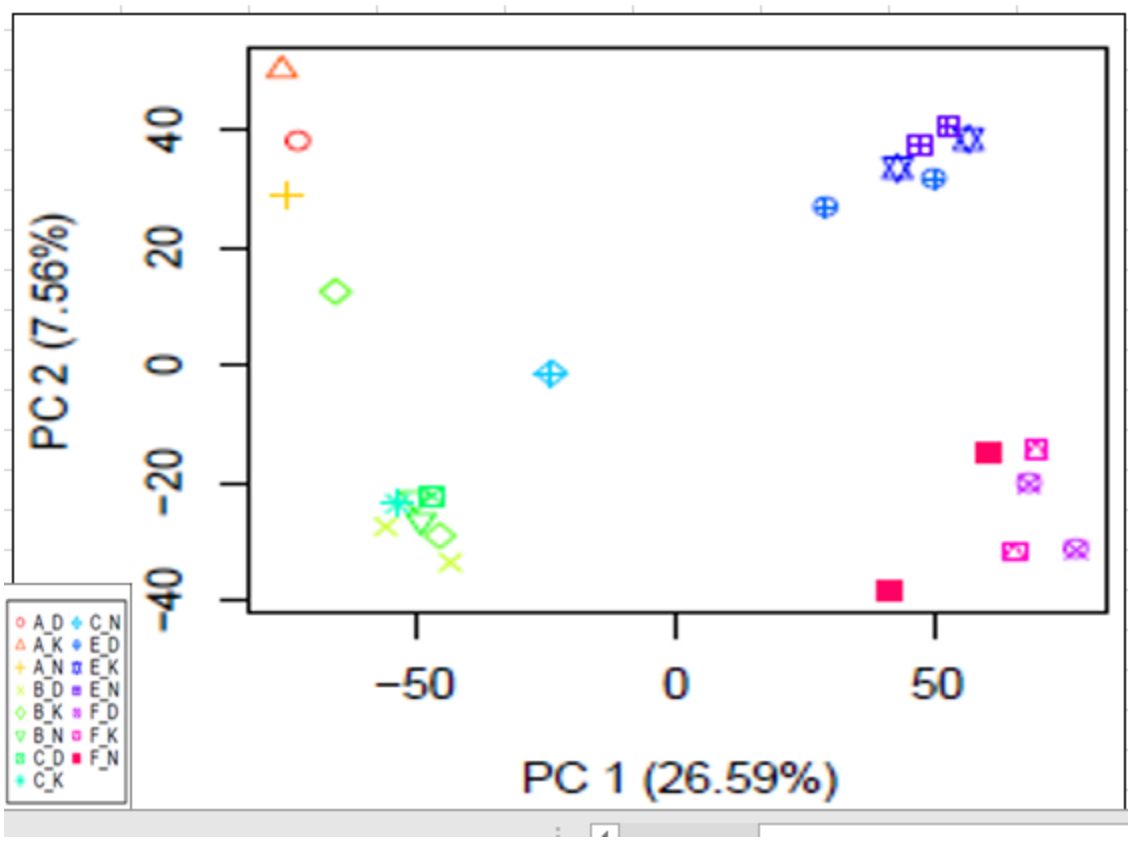

Figure 2. PCA of differential gene expression of maize kernels under different treatments. First letter refers to harvest dates, second letter refers to inoculation treatments of kernels. A – Jun 08, B & C – Jun 18 & 22, E – Jun 26, F – Jul 3. D – side needle, K – silk channel, N-none.

General characteristics of gene expression in the 24 samples

The maturity of the kernels at harvest appeared to be the greatest source of variation, as indicated by the principal component analysis of log2(read counts), re-scaled or normalized based on library sizes in Figure 2. Using a single gene as an example, at the sucrose synthase (shrunken1) locus, the read counts normalized by the TMM method (Dillies et al. 2013) at the blister stage ranged from 1,032 - 1,217, at the milk stage: 444 - 701, at the dough stage:

195 - 276, and at the dent stage: 161 – 259. This led samples to primarily be grouped and analyzed according to the stage of development, which indicated effects of any other treatments or factors should be evaluated within the context of stage of maturity.

Even with the limited number of biological replicates within the treatment sets, the DGE in response to inoculation and/or the presence of *A. flavus* were distinctive at each stage regarding specific patterns of gene ontologies, numbers of genes, and magnitude and direction of expression. A paired t-test applied to the average read counts at each stage showed no significant differences between the two inoculation methods for blister and dent samples. However, results were significant at the milk and dough stages, likely due to much fewer genes being differentially expressed, and of these, only a small percentage were up or down-regulated under both types of treatment. In the blister group, both methods of inoculation versus non-inoculation resulted in mostly up-regulation of over 50 genes, with similar fold changes between inoculation methods that often exceeded two; this despite the observation that few fungal reads were detected in the silk channel sample. This finding suggests the fungus can dramatically influence the host's gene expression, even when the fungus is only present at low levels. More noteworthy is the DGE in response to the inoculation at such an early stage of development, as fungal infection of kernels damaged (Dolezal et al. 2015) or un-damaged (Marsh and Payne 1984) have not been reported, and have not been believed to occur before milk stage. However, it is possible that early host responses to inoculum is a function of a "resistant" genotype that would not be observed in a susceptible genotype.

In total, there were 685 unique transcripts DE between inoculated samples versus

non-inoculated samples over all stages of maturity within the bounds of $p \leq .05$, FDR $\leq .10$, and a fold change of 2 ($\log2FC \geq 1$). The likelihood ratio test was applied to each comparison which permitted a ranking of DEGs and provided a p-value and FDR. In Table 12, the 50 genes most significantly differentially expressed at the blister stage under both methods of inoculation are presented, excluding those with uncharacterized gene products. Forty-four of the 50 genes were significantly DE under *both* inoculation treatments, with similar fold changes and direction. In Table 13, DGE of kernels at stages milk to dent is presented in a similar manner; the largest group of significantly DEGs was found in the side-needle inoculated dent kernels, but no more than the top fifty genes were subjected to analysis.

Over all stages of development, aflatoxin levels were higher in the side needle inoculated samples (Table 11), and there were much higher levels of fungal reads in certain side needle samples.

Table 12. Differential gene expression fold change (FC) in inoculated kernels at the blister stage

Gramene IDs	Gene Product	Gene Ontology	FC(AD _ AN)	FC(AK_AN)	Classification
GRMZM2G140970	ascorbate peroxidase	strong antioxidant (removes hydrogen peroxide)	10.54	8.25	abiotic/biotic stress
GRMZM2G444748	bzip transcription factor 60	stess related TF, response to presence of chitin	20.68	25.98	abiotic/biotic stress
GRMZM2G005633	chitinase family 19	antifungal	4.69	-	abiotic/biotic stress
GRMZM2G326111	cyclophilin(peptidyl-prolyl cis-trans isomerase)	protein folding (like chaperone)	9.90	8.98	abiotic/biotic stress
GRMZM2G392863	defensin/ gamma-thionin	host defense peptide	0.20	0.14	abiotic/biotic stress
GRMZM2G112165	HSP-90	chaperone protein - assists in folding	4.12	3.66	abiotic/biotic stress
GRMZM2G050412	jasmonate-induced protein	(disease resistance)	257.77	226.95	abiotic/biotic stress
GRMZM2G011526	LRR receptor ser/thr protein kinase	protein phosphorylation and disease resistance	286.29	148.36	abiotic/biotic stress
GRMZM2G056629	aldose 1-epimerase	glycolysis (galactose metabolism)	0.30	0.21	carbohydrate metabolism
GRMZM2G071630	glyceraldehyde - 3 - phosphate dehydrogenase	glycolysis of glucose for energy	-	5.09	carbohydrate metabolism
GRMZM2G139300	invertase1 (cell wall)	hydrolysis of sucrose	3.86	8.32	carbohydrate metabolism
GRMZM2G383404	anthocyanidin 3-O-glucosyltransferase	anthocyanin biosynthesis	286.29	295.71	cellular metabolism
GRMZM2G145573	oxidoreductase, acting on NADH or NADPH	cell metabolism, biosynthesis, superoxide dismutase activity	4.00	4.45	cellular metabolism
GRMZM2G038536	ADP-ribose polymerase 3	DNA repair	-	0.24	other
GRMZM2G032145	BURP domain in cell wall protein	unknown function	3.97	3.89	other
GRMZM2G406170	BURP domain-containing protein 4-like	unknown function	4.25	4.22	other
GRMZM2G401139	cytochrome b-c1 complex subunit 8	electron transport chain in mt	3.00	2.84	other
GRMZM2G010762	early nodulin-like protein 3	cell membrane component with electron carrier activity (TAIR)	4.11	5.33	other
GRMZM5G831200	polygalacturonase	(pectin depolymerase - fruit ripening)	0.22	0.35	other
GRMZM2G021517	protein kinase	protein phosphorylation	0.17	0.14	other
ZEAMMB73_435058	elongation factor 1	protein synthesis	4.15	5.42	protein synthesis
GRMZM2G343543	elongation factor 1 alpha	protein synthesis	2.49	2.71	protein synthesis
GRMZM2G153541	elongation factor 1 alpha	protein synthesis	2.30	3.08	protein synthesis
GRMZM2G154218	elongation factor 3	protein synthesis	4.07	4.67	protein synthesis
GRMZM2G030228	ribosomal 40S	protein synthesis	7.39	4.61	protein synthesis
GRMZM2G145258	ribosomal 40S S3A3 family	protein synthesis	2.17	2.60	protein synthesis
GRMZM2G125271	ribosomal protein 40S	protein synthesis	3.85	5.30	protein synthesis
GRMZM2G104025	ribosomal protein 60S	protein synthesis	4.57	6.96	protein synthesis
GRMZM2G887054	ribosomal protein 60S	protein synthesis	4.17	4.04	protein synthesis
GRMZM2G119809	ribosomal protein 60S	protein synthesis	18.00	26.91	protein synthesis
GRMZM2G083253	ribosomal protein 60S	protein synthesis	2.31	2.29	protein synthesis

Table 12 continued.

Gramene IDs	Gene Product	Gene Ontology	FC(AD _ AN)	FC(AK_AN)	Classification
GRMZM2G113720	ribosomal protein 60S	protein synthesis	4.24	6.81	protein synthesis
ZEAMMB73_000159	ribosomal protein 60S	protein synthesis	2.49	3.10	protein synthesis
GRMZM5G820996	ribosomal protein 60S	protein synthesis	2.12	2.06	protein synthesis
GRMZM2G171181	ribosomal protein 60S	protein synthesis	2.36	3.06	protein synthesis
GRMZM2G047727	ubiquitin-ribosomal protein 60S	pre-60S ribosomal protein	2.99	2.87	protein synthesis
GRMZM2G160739	alpha zein	storage protein in endosperm	6.15	7.14	seed storage protein
GRMZM2G044625	alpha zein	storage protein in endosperm	5.51	6.92	seed storage protein
GRMZM2G044625	alpha zein	storage protein in endosperm	5.99	7.28	seed storage protein
GRMZM2G704406	alpha zein	storage protein in endosperm	4.29	3.59	seed storage protein
GRMZM2G346897	alpha zein	storage protein in endosperm	4.34	5.37	seed storage protein
GRMZM2G044625	alpha zein	storage protein in endosperm	6.06	7.40	seed storage protein
GRMZM2G397687	alpha zein	storage protein in endosperm	4.32	3.76	seed storage protein
GRMZM2G060429	gamma zein 16 kD	storage protein in endosperm	31307.34	35641.76	seed storage protein
GRMZM2G025857	late embryonic abundant protein	seed storage protein	-	0.07	seed storage protein
GRMZM2G067985	actin	cellular structure and mobility / ATP binding	2.27	2.36	structural
GRMZM2G118873	expansin gene - loosens cell walls	cell wall organization	15.74	10.09	structural
GRMZM2G108766	tubulin beta-8 chain	structural constituent of cytoskeleton	3.63	2.96	structural
GRMZM5G800112	endothelial differentiation-related factor 1	transcription regulation - binds with TATA box (Interpro)	2.52	2.73	transcription regulation
GRMZM2G129034	MADS box family (AGL2)	transcription factors for flower development	3.44	3.30	transcription regulation

Stages of Seed Development: A - blister

Method of inoculation: D - side needle, K - silk channel

† $p \leq .05$, and FDR $\leq .10$ for all entries

Table 13. Differential gene expression fold change (FC) in inoculated kernels at milk, dough and dent stages

			Fold Changes †						
Gramene IDs	**Gene Product**	**Gene Ontology**	**BD - BN**	**BK - BN**	**ED - EN**	**EK - EN**	**FD - FN**	**FK - FN**	**Classification**
GRMZM2G150256	cysteine protease	degrades proteins and aids in pathogen resistance	78.41						abiotic/biotic stress
GRMZM6G198866	metallothionein-like protein type 2	metal ion binding / detoxification	0.13						abiotic/biotic stress
GRMZM2G135978	transport inhibitor with leu rich repeat domain	proteasomal degradation and auxin-regulated transcription	0.01	0.01					other
N/A	18S ribosomal RNA gene, partial sequence	protein synthesis	0						protein synthesis
GRMZM2G433162	amino acid permease 3-like	amino acid transport into the cell		9.75					other
GRMZM2G108277	mitosis protein dim1 with thioredoxin-like fold	redox signaling in respiration		245.52					signal transduction
GRMZM2G057093	chitinase 2 family 18	chitin degrading, antifungal			38.64a	60.42			abiotic/biotic stress
N/A	lncRNA drought responsive	regulatory, stress response			0.02	0.02			abiotic/biotic stress
GRMZM2G334181	Protein kinase	related to salt stress/antifungal			49.25a	84.39			abiotic/biotic stress
GRMZM2G007757	beta-ketoacyl synthase family protein	fatty acid synthesis			0				cellular metabolism
GRMZM2G051103	calcium binding interacting protein kinase family protein	signal transduction through calcium binding			0.38				signal transduction
GRMZM2G179792	phospholipase D family protein	lipid signaling enzyme			3.3				signal transduction
GRMZM2G024996	glycine-rich cell wall structural protein-like	structural			5.07				structural
GRMZMG138178	RNA polymerase II	transcription mediator			64.11				transcription regulation
GRMZM2G156861	lipoxygenase1 (lox 1)	catalyzes the hydroperoxidation of lipids / antifungal				17.93			abiotic/biotic stress
GRMZM2G112792	L-gulonolactone oxidase	biosynthesis of ascorbic acid/ photosynthesis and cell growth				5.92	11.35	5.79	cellular metabolism
GRMZM2G349749	patatin/phospholipase A2-related	lipid acyl hydrolase that degrades polyhydroxyalkanoate				11.43	5.03		cellular metabolism

Table 13. Continued

Gramene IDs	Gene Product	Gene Ontology	BD - BN	BK - BN	ED - EN	EK - EN	FD - FN	FK - FN	
GRMZM2G106730	nodulin-like protein, transporter (major facilitator family)	transport of nutrients, solutes,aa or hormones							other
GRMZM2G090245	auxin-binding protein ABP20 precursor/Cupin 1	seed storage protein				2.65	3.44		seed storage protein
GRMZM2G172204	beta glucosidase jasmonate-induced aggregating factor1	jasmonate is related to stress response					8.87	5.21b	abiotic/biotic stress
GRMZM2G042639	GST activity safener induced1	protein transport / binding of toxins					2.19	1.98	abiotic/biotic stress
GRMZM2G127251	hydroxycinnamoyl transferase3	lignin pathway					3.28		abiotic/biotic stress
GRMZM5G822593	lipoxygenase 8, PLAT domain	hydroperoxidation of lipids / antifungal					6.47	3.51b	abiotic/biotic stress
GRMZM2G152638	lncRNA	drought responsive					3.68	2.28	abiotic/biotic stress
GRMZM2G036048	O-methyltransferase	lignin biosynthesis, stress tolerance and disease resistance					5.4		abiotic/biotic stress
GRMZM2G127418	O-methyltransferase	lignin biosynthesis, stress tolerance and disease resistance					6.81	3.75b	abiotic/biotic stress
GRMZM2G103055	alpha amylase	starch digestion to glucose and maltose					3.82		carbohydrate metabolism
GRMZM2G138468	alpha amylase3	starch digestion to glucose and maltose					48.1	34.02	carbohydrate metabolism
GRMZM2G031660	beta-glucosidase	glucose generating hydrolase					0.33		carbohydrate metabolism
GRMZM2G410916	glycosyl transferase	carbohydrate metabolism					0.42		carbohydrate metabolism
GRMZM2G394450	invertase (Ivr1)	hydrolysis of sucrose					72.97		carbohydrate metabolism
GRMZM2G394450	invertase (Ivr1)	hydrolysis of sucrose					5.96		carbohydrate metabolism
GRMZM2G153536	amino-acid aminotransferase (branched-chain)	amino acid bisynthesis					2.47		cellular metabolism
GRMZM2G359298	copper amine oxidase	metabolism of amino acids					0.41		cellular metabolism
GRMZM2G170400	cyclopropane-fatty-acyl-phospholipid synthase	fatty acid synthesis					6.32		cellular metabolism

Table 13. Continued

Gramene IDs	Gene Product	Gene Ontology	BD - BN	BK - BN	ED - EN	EK - EN	FD - FN	FK - FN	other
GRMZM2G023847	blue copper protein	redox process in photosynthesis					6.11		other
GRMZM2G336448	carbohydrate transporter	membrane transporter of carbohydrates					1.82		other
GRMZM2G077809	copine	Ca2+-dependent phospholipid-binding proteins					0.01		other
GRMZM2G332562	dicarboxylic acid transport	amino acid transporter					2.22		other
GRMZM2G076239	hydroxyacid oxidase 1 (glycolate oxidase)	photorespiration					0.3		other
GRMZM5G842071	laccase 1 (LAC1) gene, a multicopper oxidase	a blue copper oxidase					2.69		other
GRMZM2G061527	leucine-rich repeat domain	involved in many protein-protein interactions					3.18		other
GRMZM5G889138	NADH dehydrogenase subunit 7	cellular respiration					5.27		other
GRMZM2G037411	pectinesterase	degrades pectin, fruit-ripening					0.38		other
GRMZM2G025459	protein kinase 5'-AMP-activated subunit beta-1	cellular energy					2.95		other
GRMZM2G073114	ripening-related protein 3-like	fruit ripening					134.21		other
GRMZM2G029506	peptidase activity ATP-dependent	protease					1.98		protein degradation
GRMZM2G124684	proteinase - Aspartic	catalytic protease enzyme					506.74		protein degradation
GRMZM2G474534	ribosomal protein 30S , chloroplastic	protein synthesis					46.85		protein synthesis
GRMZM2G044625	alpha zein	seed storage protein					0.37		seed storage protein
GRMZM2G044625	alpha zein	seed storage protein					0.33		seed storage protein
GRMZM2G397687	alpha zein	seed storage protein					0.4		seed storage protein
ZEAMMB73_324839	cupin	seed storage protein					17.72		seed storage protein
GRMZM2G060429	gamma zein 16 kDa	seed storage protein					0.25		seed storage protein

Table 13. Continued

Gramene IDs	Gene Product	Gene Ontology	BD - BN	BK - BN	ED - EN	EK - EN	FD - FN	FK - FN	seed storage protein
GRMZM2G122228	protein phosphatase homolog8	signal transduction					0.36		signal transduction
GRMZM5G822829	anthocyanin pathway r1 (colored1)	transcription factor in anthocyanin biosynthesis						0.47	transcription regulation
GRMZM2G146283	endosperm-specific prolamin box binding factor (PBF) zinc finger	binds basic leucine zipper transcript activator *opaque2*					0.4	0.46	transcription regulation
GRMZM2G006676	ligand-dependent nuclear receptor activity	transcription regulation					2.27		transcription regulation
GRMZM2G359952	MADS2	transcription factors for flower development					0.3		transcription regulation
GRMZM2G172327	MYB-transcription factor 14	transcription factor for a stilbene phytoalexin as stress response					0.36		transcription regulation
GRMZM2G015534	opaque endosperm2 Basic leucine-zipper C terminal	transcription factor for zeins and other proteins					0.49		transcription regulation
GRMZM2G157219	trihelix-transcription factor 1	transcription factor in seed development					0.01		transcription regulation
GRMZM2G383404	anthocyanidin 3-O-glucosyltransferase	anthocyanidin biosynthesis					0.09		other
GRMZM2G430755	cation/H(+) antiporter 15-like	cell membrane transport					2.18		other
GRMZM2G076343	legume lectins beta domain containing protein	carbohydrate binding protein					2.42		other
GRMZM2G168474	O-glucosyltransferase 2 (cis-zeatin)	protein glycosylation and cytokinin activation					2.02		other
GRMZM2G017013	protein binding (in apoptosis)	programmed cell death					0.18		other
GRMZM2G164787	polyubiquitin containing 7 ubiquitin monomers	protein degradation and recycling					69.17		protein degradation
GRMZM2G016323	ubiquitin carboxyl-terminal hydrolase 23	protein degradation and recycling					0.02		protein degradation
GRMZM2G075104	ubiquitin conjugation factor E4	protein degradation and recycling					49.86		protein degradation
GRMZM2G000741	GTPase Protein-synthesizing	chloroplast protein synthesis					0.01		protein synthesis

Stages of Seed Development: B - milk, E - dough, F - dent

Method of inoculation: D - side needle, K - silk channel

† $p \leq .05$, and FDR $\leq .10$ for all entries with exceptions listed below:

a FDR = .13

b. $.13 \leq$ FDR $\leq .20$

DEGs previously associated with injury or pathogenesis and their function in A. flavus infection

A total of 16 DE protein-coding genes were identified under one or both inoculation treatments in this study that have been previously associated with a response to injury and/or presence of a pathogen in relevant other studies (Table 14). These primarily increased under inoculation but one, gamma-thionin, decreased (Figure 3). Given the corroborating evidence of our study, these 16 DE protein-coding genes are worth discussing in more detail, and will be grouped according to a function highlighted in this study.

Antifungal group: Chitinases are often implicated in plant fungal defenses and belong to the second largest group of pathogenesis-related proteins (PR). They are included in families 18 and 19 of glycoside hydrolases, and catalyze chitin degradation in the fungal cell wall (Ferreira et al. 2007). Some classes are mainly chitin-binding, and thus inhibit fungal growth by disrupting cell polarity when bound to the fungal cell wall. In this study, GRMZM2G005633 of family 19 was up-regulated by over four-fold in the side-needle inoculated kernels at blister stage, while in the dough stage both the silk channel and side needle inoculations were associated with up-regulation of about 32-fold in GRMZM2G057093 of family 18. The former was assigned to the maize genome region of bin 10.04 (Hawkins et al, 2015) while the latter was assigned to bin 1.08 by MaizeCyc, a network of metabolic pathways delineated in B73 (Monaco et al. 2013).

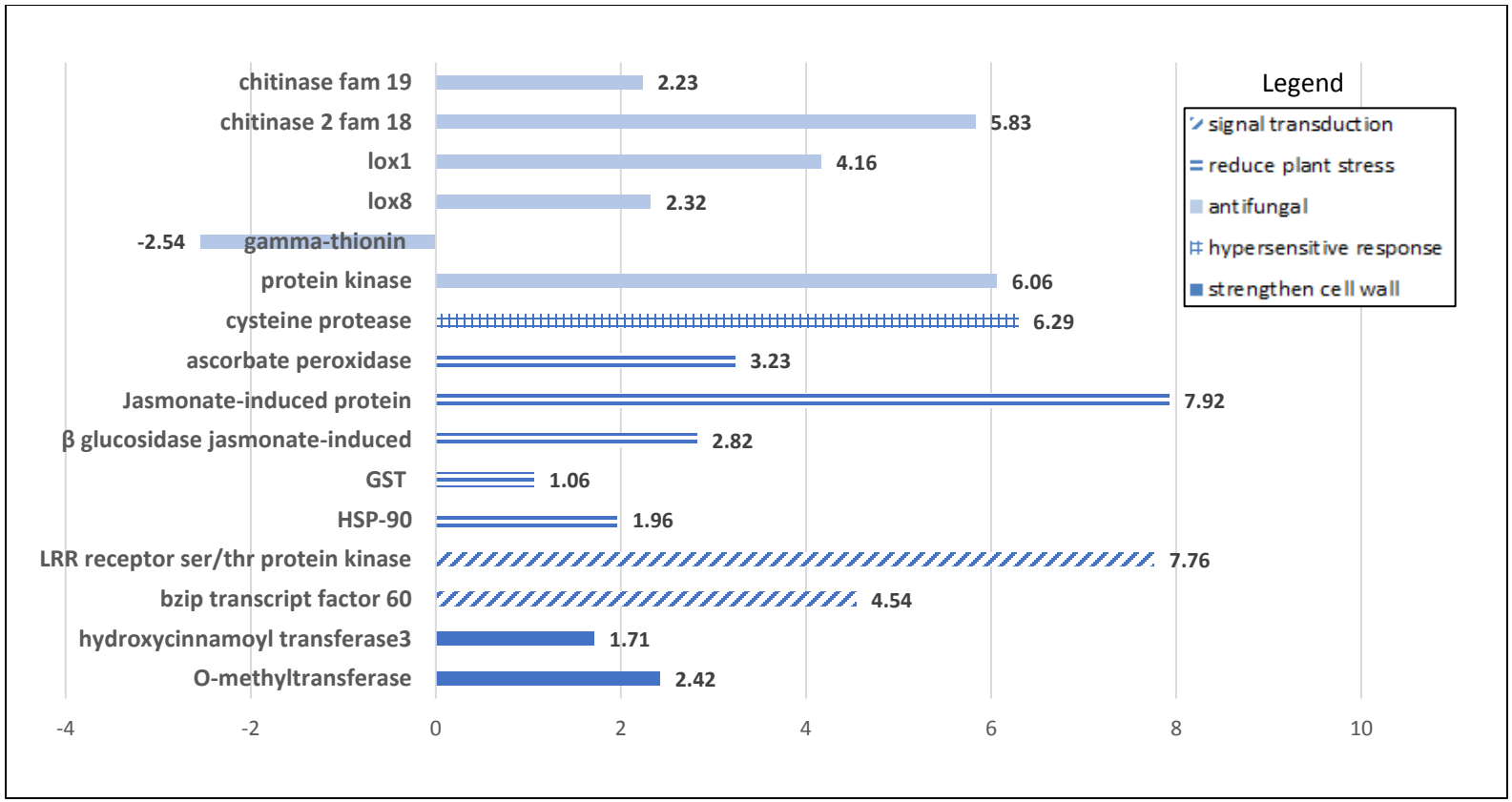

Figure 3. Differential gene expression represented by log2(fold changes) at different stages of kernel maturity that have been previously associated with abiotic stress response, at $p \leq .05$, and $FDR \leq .10$.

Table 14. Differentially expressed genes associated with presence of pathogen

Gene	Gramene #	Biological Process	References
Chitinase family 19	GRMZM2G005633	antifungal, degrades fungal cell walls	1,2,3,4
Chitinase family 18	GRMZM2G057093	antifungal, degrades fungal cell walls	1,2,3,4
Lipoxygenase 1 (LOX1)	GRMZM2G156861	stress or wound induced	5,6,7
Lipoxygenase 8 (LOX8)	GRMZM5G822593	stress or wound induced	5,6,7
Flower-specific γ-thionine	GRMZM2G392863	fungal inhibition through ion efflux mechanism	3,8,9
Protein kinase (related to salt stress)	GRMZM2G334181	antifungal	2,3,8
Cysteine protease	GRMZM2G150256	hypersensitive response	8,10,11,12
Ascorbate peroxidase	GRMZM2G140970	strong antioxidant, removes hydrogen peroxide	1,8,13
Jasmonate-induced protein	GRMZM2G050412	stress response, disease resistance	5,6,14
Beta glucosidase jasmonate-induced aggregating factor 1	GRMZM2G172204	stress response, disease resistance	5,6,14
Glutathione-S-transferases (GST)	GRMZM2G042639	detoxification of toxic substances	2,3,8,15,16,17
Hsp90	GRMZM2G112165	reduce plant stress, molecular chaperone	18,19,
Receptor-like ser/thr kinases with LRR	GRMZM2G011526	signaling in pathogen recognition	2,3,8,20
Bzip transcription factor 60	GRMZM2G444748	transcription factor regulating response to chitin	21
Hydroxycinnamoyl transferase	GRMZM2G127251	lignin biosynthesis	2,3,18
O-methyltransferase	GRMZM2G036048	lignin biosynthesis	2,3,18

Table 14.
Continued

1	Shigeoka et al., 2002	Regulation and function of ascorbate peroxidase isoenzymes
2	Dolezal et al., 2015	*Aspergillus flavus* infection induces transcriptional and physical changes in developing maize kernels
3	Luo et al., 2011	Transcriptional profiles uncover *Aspergillus flavus*-induced resistance in maize kernels
4	Hawkins et al., 2015	Characterization of the maize chitinase genes and their effect on *Aspergillus flavus* and aflatoxin accumulation resistance
5	Tang et al., 2015	Using genome-wide associations to identify metabolic pathways involved in maize aflatoxin accumulation resistance
6	Christensen et al., 2013	The maize liposygenase, ZmLOX10, mediates green leaf volatile, jasmonate and herbivore-induced plant volatile production for defense against insect attack
7	Mideros et al., 2014	Quantitative trait loci influencing mycotoxin contamination of maize: analysis by linkage mapping, characterization of near-isogenic lines and meta-analysis
8	Jiang et al., 2011	Expression analysis of stress-related genes in kernels of different maize (Zea mays L.) inbred lines with different resistance to aflatoxin contamination
9	Ferreira et al., 2007	The role of plant defense proteins in fungal pathogenesis
10	Solomon et al., 1999	Involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants
11	Lampl et al., 2013	Set-point of RD21 protease activity by ATSerpin1 controls cell death in Arabidopsis
12	Shindo et al., 2012	A role in immunity for Arabidopsis cysteine protease RD21, the ortholog of the tomato immune protease C14
13	Pechanova et al., 2011	Proteomic analysis of the maize rachis: Potential roles of constitutive and induced proteins in resistance to *Aspergillus flavus* infection and aflatoxin accumulation
14	Chaudry et al., 1994	The barley 60 kDA jasmonate-induced protein (JIP60) is a novel ribosome-inactivating protein
15	Holt et al., 1995	Characterization of the safener-induced glutathione S-transferase isoform II from maize
16	Fortunato et al., 2015	Changes in the antioxidant system in soybean leaves infected by *Corynespora cassiicola*
17	Wisser et al., 2011	Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a GST gene

Table 14.
Continued

18	Kelley et al., 2012	Identification of maize genes associated with host plant resistance or susceptibility to *Aspergillus flavus* infection and aflatoxin
19	Xu et al., 2012	Heat Shock Protein 90 in Plants: Molecular Mechanisms and Roles in Stress Responses
20	Afzal et al., 2008	Plant receptor-like serine threonine kinases: roles in signaling and plant defense
21	Libault et al., 2007	Identification of 118 Arabidopsis transcription factor and 30 ubiquitin-ligase genes responding to chitin, a plant-defense elicitor

Hawkins et al. (2015) did not find that chitinase (GRMZM2G005633), which they identified in their QTL mapping populations of hybrids derived from susceptible and resistant parents, contributed to any phenotypic effect with respect to aflatoxin contamination resistance. This was possibly due to post-translational modifications of the chitinase by a fungal protease (Naumann, Wicklow, and Kendra 2009; Naumann and Wicklow 2010). Chitinase 2 (GRMZM2G057093), significantly DE in this study, has not appeared in the genetic mapping populations discussed previously, likely because it was not segregating between the population parents (Hawkins et al. 2015). Here in Tx772, this gene was expressed in the dough samples, and may have been instrumental in preventing the levels of infection from more quickly reaching those of the blister or dent stages, according to the relative numbers of fungal reads, as will be discussed later.

Lipoxygenase resistance to pathogens has often been difficult to determine, including the two up-regulated in this study, LOX1 (GRMZM2G156861) under silk channel inoculation in dough kernels by 16 fold, and LOX8 (GRMZM5G822593) by more than four-fold in the dent samples (Fountain et al. 2015). Yet there is evidence from QTL studies (Mideros et al. 2014) and a DGE study (Christensen et al. 2013) that these two genes contribute to resistance of aflatoxin contamination. Furthermore, in a genome-wide association study to identify metabolic pathways contributing to resistance to aflatoxin contamination in maize, genes for both LOX1 and LOX8, contributed a highly significant positive effect (Tang et al. 2015). LOX8, among other lipoxygenases, contributes to the biosynthesis of the hormone jasmonate (JA) (Christensen et al. 2013), and this is the key hormone associated with an incremental decrease to levels of aflatoxin in a GWAS panel (Tang et al. 2015).

One protein belonging to a multi-functional class of defense proteins, flower-specific γ-thionines (GRMZM2G392863), was down-regulated in the inoculated blister samples. This was the only DE protein identified as antifungal and protective against insect pests to be downregulated under inoculation (Lay et al. 2003) in this study. Another unnamed protein, (GRMZM2G334181) related to a protein kinase that responds to salt stress (Zhang et al. 2009), and shows homology to an antifungal protein with protease inhibitory activity (Sawano et al. 2007) as aligned in the Pfam database, was similarly DE in the dough stage under both treatments by around six-fold.

Hypersensitive type: There are many normal metabolic processes that can lead to the production of reactive oxygen species (ROS) that are harmful to the cell, but abiotic and biotic stresses may lead to an excess of ROS (Shigeoka et al. 2002). If ROS levels exceed a certain threshold programmed cell death (PCD) or apoptosis can be activated in a hypersensitive response to an invading pathogen (Solomon et al. 1999). Cysteine protease (GRMZM2G150256), which is activated by high levels of ROS (Solomon et al. 1999), was highly up-regulated in side needle inoculated milk stage samples. Plants also have protease inhibitors to limit the PCD, but none were DE in this study.

Stress response group: In contrast to the effects of proteases, peroxidases in the plant as well as in the fungus are protective against the damaging effects of ROS arising from increased levels of H_2O_2 in response to pathogenic attacks and other factors (Shigeoka et al. 2002), and an ascorbate peroxidase (APX) gene (GRMZM2G140970) was up-regulated by about 8-fold in both blister samples. Experiments on transgenic antisense tobacco with reduced APX infected with the bacterium *Pseudomonas syringae* resulted in elevated cellular

H_2O_2 levels that led to enhanced cell death (Mittler et al. 1999). However, it must be considered that H_2O_2 has beneficial roles as well as detrimental ones that must be balanced in a regulatory system (Shigeoka et al. 2002); this would explain why gene expression of some peroxidases are down-regulated instead of up-regulated under similar experimental conditions.

Jasmonate is a plant hormone noted for increasing resistance to necrotrophs such as *A. flavus* (Glazebrook 2005). In this study two genes associated with proteins described as "induced by jasmonate" were up-regulated, one in the blister inoculated samples (GRMZM2G050412) with large positive fold changes for side needle and silk channel treatments, and the other a beta glucosidase jasmonate-induced aggregating factor1 (GRMZM2G172204) in the dent samples with smaller fold changes.

Glutathione-S-transferases (GST) has a role in detoxifying toxic substances encountered during biotic and abiotic stress, and has been moderately correlated with resistance to a number of maize pathogens (Wisser et al. 2011). Gene expression for a protein with GST activity (GRMZM2G042639) was upregulated about two-fold under both inoculation treatments in the dent samples.

Certain heat shock proteins such as Hsp90 are involved in disease and pest resistance besides acting as molecular chaperones to regulate and maintain proper protein conformations (Xu et al. 2012; Liu et al. 2004). Kelley et al. (2012) reported that Hsp90 was up-regulated in resistant Mp313E over susceptible Va35 in response to *A. flavus* inoculation. In this study Hsp90 GRMZM2G112165) was differentially expressed in the inoculated blister samples about four-fold.

Signal transduction group: The complex roles of plant receptor-like serine threonine kinases with a leucine rich repeat domain have often been related to their activities in signaling and plant defense (Afzal, Wood, and Lightfoot 2008; DeYoung and Innes 2006). The results of one study suggested that two wheat leucine rich repeat– receptor-like kinases (LRR-RLKs) significantly enhanced resistance to powdery mildew caused by the fungus, *Blumeria graminearum* when inoculated into wheat (Chen et al. 2016). Another study discussed the mapping of a LRR-RLK gene to a locus in the barley genome that has been shown to be effective in providing rust resistance in barley stems, which shares homologies with genes of similar functions in maize, rice and tomato (Brueggeman et al. 2002). In the blister samples, the LRR receptor ser/thr protein kinase (GRMZM2G011526) experienced an approximately 64-fold increase for both treatments.

Bzip transcription factor 60 (GRMZM2G444748), identified in *Arabidopsis thaliana* as AT1G42990 has shown significant DGE under pathways unique in the response to chitin elicitation (Libault et al. 2007; Zhang et al. 2002) that were initially independent of three stress hormones: ethylene, jasmonic acid and salicylic acid. Bzip transcription factor 60 was DE by more than 20-fold in both the silk and side needle blister samples, Table 12.

Lignin biosynthesis group: In host plants a build-up of lignin has been associated with resistance to fungal growth, providing a first line of defense against pathogen invasion as it strengthens the cell wall against mechanical pressure arising from fungal appressoria attempting penetration. Lignin is synthesized from phenylpropanoid hydroxycinnamyl alcohols, (Ebrahim, Usha, and Singh 2011), and the enzyme hydroxycinnamoyl transferase (GRMZM2G127251) was up-regulated by more than two-fold in the dent side-needle

inoculated samples. Another enzyme involved in the lignin biosynthesis pathway, O-methyltransferase (GRMZM2G127418, GRMZM2G036048), was up-regulated by more than four-fold in both side needle and silk channel samples at the dent stage. Regarding hydroxycinnamoyl transferase3 involved in lignin biosynthesis, (Kelley et al. 2012) reported that a related gene, cinnamoyl-CoA reductase was significantly expressed in the susceptible maize line, Va35 upon inoculation with *A. flavus*. O-methyltransferase is also essential in lignin biosynthesis, and a mutation in that gene produces the *brown midrib3* phenotype, which modifies and reduces lignin content in the stems and roots, making the stems more digestible as a forage crop (Vignols et al. 1995). The effects of RNA-mediated silencing to inhibit the former enzyme, which has a key position in the phenylpropanoid pathway in the formation of lignin, arrested early development of Arabidopsis plants (Hoffman et al., 2004). In transgenic lines of alfalfa (*Medicago sativa* L.) down-regulation of enzyme activity from 15-50% by RNA-mediated silencing resulted in significant stunting, reduced biomass, and delayed flowering (Shadle et al. 2007). Therefore, knocking down enzymes that are known to be key to lignin biosynthesis to test disease resistance would likely have undesirable side effects. However, the correlation between levels of phenolic compounds that are incorporated into lignin following inoculation with the fungus as was done with peanuts, another crop subject to aflatoxin contamination, has been measured (Liang et al., 2006). Not only was there a significant negative correlation between infection rate and lignin abundance following peanut inoculation, but resistant genotypes required much less time to reach maximum levels of key enzyme activity to metabolize lignin precursors than susceptible types. Other studies have shown this as well (Fajardo et al. 1994a, 1994b; Liang, Luo, and Guo 2006)

Other classes of genes DE not directly related to pathogenic response

In addition to the proteins described above for which there is some evidence of an antifungal effect, two other classes of genes that were differentially expressed in this and previous studies should be mentioned.

Five different alpha zein genes in the blister stage were up-regulated in the inoculated samples compared with the non-inoculated ones with fold changes in the range of 3.3 – 7.4 (Table 12 and Appendix 8), as were 15 genes related to translation including ribosomal proteins and elongation factors. In addition, a 16kD gamma zein was extremely up-regulated in the same comparison, making it the most highly up-regulated gene. Coincident with the up-regulation of the alpha zein genes, twelve genes coding for ribosomal proteins along with four coding for elongation factors were up-regulated at the blister stage as well. Two previous studies on transcriptional patterns in maize identified the *opaque2* transcription factor, which through a regulatory network, affects the expression of certain alpha zein proteins, together with certain ribosomal genes and elongation factors (Li et al. 2015; Hunter et al. 2002). Although DE of the *opaque2* was not detected at this stage in the current study, there is reasonable evidence that it was expressed in the blister samples to permit alpha zein gene expression.

In the dent stage, however, all the alpha zeins and the same gamma zein were down-regulated, the former by about 60%, and the latter by 75% (Table 13). The same pattern of down-regulation for all zeins was observed in the study of DGE on field inoculated ears at different stages of maturity combined (Dolezal et al. 2015). In addition, two transcription factors that regulate zein expression were also down-regulated including *opaque2*

(GRMZM2G015534) by 50%, and endosperm-specific prolamin box binding factor (PBF) zinc finger (GRMZM2G146283) by 60%.

An important consideration in the novel up-regulation of zeins is that TX772 has a vitreous (i.e. hard, flinty) endosperm, while most if not all of the germplasm tested in comparable DE studies were dent type (softer, more floury) endosperm (not to be confused with the dent kernel development stage). Dent types include the resistant inbred line Mp313E, which is a yellow dent type developed from Tuxpan (Scott and Zummo 1990), and another inbred line derived from it, Mp715 (Williams and Windham 2001). When vitreous and floury endosperms were compared for protein and starch composition, the increase in alpha zeins (twice as much in flint compared to floury) as well as the arrangement and size of starch granules contributed to the hardness of the kernel (Gayral et al. 2016). Vitreous compared to softer dent type endosperm has been positively correlated with resistance to ear rot and aflatoxin contamination (Betran, Isakeit, and Odvody 2002; Llorente, Betrán, et al. 2004; Darrah et al. 1987). Perhaps up-regulating zein genes as found at the blister stage in response to infection is one way that Tx772 builds up resistance to colonization by the fungus, as evidenced by greater expression of these genes in inoculated samples. Yet, some infection did occur in the samples harvested at the dent kernel development stage, and at that point zein gene expression in the non-inoculated kernels was two to three-fold higher than those that were inoculated. In the (Dolezal et al. 2015) study, infected kernels of susceptible B73 (a softer dent kernel type) had lost much of the zein-filled hard endosperm, and with it most of the cells still capable of producing the protein, and were replaced by starchy endosperm, by maturity. In our study, most of the kernels up to the dent stage appeared to be

intact, while zein gene expression was suppressed in inoculated kernels at this more advanced stage.

The second class of genes pertained to proteins that are known to increase free hexose levels, as observed by (Dolezal et al. 2015). In the current study we noted up-regulation of invertase cell wall1 (GRMZM2G139300) and invertase1 (GRMZM2G394450) in the blister and dent samples, and two alpha amylase genes, (GRMZM2G103055) and (GRMZM2G138468), the latter of which was up-regulated by over 32- fold in the silk channel and side needle inoculated samples compared to the non-inoculated ones. A recent study on expression profiling of 267 unigenes in a mapping population derived from a cross between an aflatoxin contamination susceptible parent and a resistant parent revealed many genes involved in the synthesis and hydrolysis of starch and sugar mobilization were highly expressed (Dhakal et al. 2017), and others related this to providing energy and/or precursors of lignin and phytoalexins used in the defense response (Bolton et al. 2008; Granot, David-Schwartz, and Kelly 2013; Shu et al. 2015; Dolezal et al. 2013). Agrios (2005) explained that when plants are infected by pathogens, the rate of respiration is up-regulated, which often translates to an increase in glycolysis. In more resistant plants, respiration increases more quickly to provide the abundant source of energy needed by its defense mechanisms. In the blister group, glyceraldehyde-3-phosphate dehydrogenase and an oxidoreductase, which are catalysts in the conversion of glucose to energy through their acting on NADH or NADPH, (Sirover 2014; Gani et al. 2016) were also up-regulated by about four-fold (Table 12). In the dent development stage group, two enzymes related to cellular respiration, NADH dehydrogenase subunit 7 and protein kinase 5'AMP-activated were up-regulated as well,

Table 13. Thus, our results, involving up-regulation of genes producing or utilizing simple sugars at the expense of those making starch, align with previous observations related to energy production, but not those related to the biosynthesis of lignins.

At the same time, some have suggested (Dolezal et al. 2015; Govrin and Levine 2000) that fungal pathogens alter the host plant's metabolism to secure their own nutrition, which could certainly be the case in increasing the levels of free hexoses. In this study, fungal genes for three enzymes contributing to glycolysis were expressed in either blister or dent samples including enolase, fructose-biphosphate aldolase and glyceraldehyde 3-phosphate dehydrogenase. In addition, fungal endoglucanase was expressed, which acts as a cellulase to degrade cell walls.

Fungal sequences show high correlation with maize DEGs and aflatoxin level

Numbers of fungal reads detected above a certain threshold (1e -20) are also listed in the Table 11 (e) and (f). Fungal cDNA sequences of mainly *A. flavus*, in addition to others identified by BLASTn (Johnson et al. 2008) such as *Aspergillus orzyae*, and *Fusarium verticillioides*, were detected especially at the blister and dent stages. At the dent stage, the non-inoculated samples had what appeared to be some natural contamination from the field. Although numbers were still significantly lower than those of the inoculated samples, this is consistent with our expectation of corn development in Texas given the large pool of *A. flavus* inoculum in the field; there is currently no way to our knowledge to eliminate all natural infection under field conditions. The correlation between number of DEGs and fungal reads for each of the eight treatment groups was 0.57, but increases to 0.88 if excluding the paucity of fungal reads detected in the blister silk channel sample which had a significant

number of maize DEGs. The range of aflatoxin was 0 to 137 ng g^{-1}, and correlated with the distribution of fungal reads at r = .65. Levels of aflatoxin in this study (Table 11 (g)) were relatively low at the time points measured, but levels are highly subject to environmental factors (Payne 1992), metabolic state of the kernels (Jiang et al. 2011), the state of the fungus (Jayashree and Subramanyam 2000), and the length of time since infection (Scott and Zummo 1994; Betran and Isakeit 2004). Mideros et al. (2009) previously showed that qPCR of the *A. flavus* internal transcribed spacer 1 (ITS1) often closely correlated to aflatoxin. Our finding suggests that overall transcript level might also be a promising measure for *A. flavus* contamination.

Characterization of fungal genes expressed

Fungal genes were identified from the samples for which at least ten reads could be assigned to a given transcript at a certain stage of maturity (Appendix 9). At the milk stage, many of the fungal loci had less than ten reads, and so were not represented in this table. There were no transcripts expressed in the kernels inoculated by silk channel that were not also expressed in the side needle samples. A few fungal transcripts, especially at the dent stage were also lowly expressed in the non-inoculated samples as well as those mentioned previously. Loci of proteins or noncoding RNAs for which a specific product has not been characterized and named, were assigned to the "uncharacterized" transcripts category; these represented about 16% of total fungal transcripts.

The genes for the 40S and 60S ribosomal proteins greatly outnumbered all other genes at the blister, dough and dent stages at 58%, 8% and 36% of total transcripts at each stage respectively. At all stages, at least one stress response gene in the fungus was

expressed. None of the 25 genes directly involved in the biosynthesis of the secondary metabolite aflatoxin were detected in this study (Yu et al. 2004; Ehrlich, Yu, and Cotty 2005). Among the stress-related transcripts, the presence of fungal superoxide dismutase in the dent stage samples indicated a need reduce the levels of ROS in the kernels, which are believed to be contributory to the production of aflatoxin (Jayashree and Subramanyam 2000; Fountain et al. 2016). In addition, presence of the CpcA expressed in the dent samples has been labeled a "cross pathway control" transcription factor, due to evidence that it controls transcription factors directly regulating production of fungal secondary metabolites, such as glioxin in *Aspergillus fumigatus*, or sirodesmin PL in the plant pathogen *Leptosphaeria maculans* (Desm.) (Elliott et al. 2011). One other interesting gene expressed, in this case in the blister kernels was ceratO-platanin, which is an extracellular secretory protein produced during kernel colonization (Dolezal et al. 2013). This phytotoxin elicits a response to infection that helps establish and maintain disease in the host plant.

3.4 Conclusion

RNA-Seq with *de novo* transcriptome assembly served to illuminate different patterns of differential expression among the four stages of maturity in maize kernels and identify the DE of many genes in maize kernels in response to field inoculation with *Aspergillus flavus*. Both the silk channel (non-wounding) and the side-needle technique (wounding) were effective in establishing *A. flavus* fungal infections as evidenced by the detection of fungal reads and aflatoxin levels in inoculated samples and producing similar DGE in magnitude and direction at each stage of maturity, even with the limited number of replications. Sixteen of the DGEs identified had been previously associated with a resistance response to the

presence of a pathogen or tissue damage, and certain others pertained to carbohydrate metabolism and energy production to support the defense response. Any future work in differential gene expression should critically consider the development stage of the seed when evaluating significant differences among genotypes or treatments. In the case of flint endosperm types in maize, we have provided some evidence of the contribution of alpha zeins can make to resistance to infection and levels of mycotoxin contamination. The complexity of biology and especially gene network analysis means that a single study can often not be definitive, and a body of evidence must be built, so it is important that here we both confirmed 16 previously implicated genes, and identified additional genetic pathways for future investigation.

Testing this further, more extensive DGE studies beyond this one is recommended, preferably on endosperm tissue alone collected at several time points with additional replications. This would provide opportunity to confirm that up-regulation of zein genes in the early stages is a novel feature of germplasm such as Tx772. An important addition would be to apply the same tests in a common garden to a well-known susceptible inbred such as B73 or Va35 to determine which genes are most likely to contribute to aflatoxin resistance in the same environments, and enable more direct comparisons with the results of similar studies

3.5 Methods and Materials

Inoculum preparation

Inoculum was prepared from the *A. flavus* isolate NRRL 3357 grown on sterilized corn kernels. The conidia were washed off and purified by repeated sedimentation through

centrifugation at 4^0C to obtain a final spore concentration of 10^7mL^{-1} (Wahl et al. 2017). The same inoculum was used for silk channel and side needle inoculation.

Field inoculation and practices

Replicate samples of maize inbred line TX772 (Llorente, Betrán, et al. 2004) were grown in College Station in 2012 and subjected to one of three treatments: 1. Three ml of inoculum down the silk channel (Zummo and Scott 1989), 2. Three ml of inoculum by side needle (Buckley, Williams, and Windham 2006b), or 3. no inoculation at 10 days after pollination (DAP). Ears were harvested at the following stages of maturity, typically around dusk: 1. blister 2. milk/early dough 3. late dough and 4. early dent. Since the milk and early dough stages were only four days apart, these samples were analyzed as one group named "milk". All kernels cut from each ear were flash frozen at harvest at -80C, ground with a mortar and pestle, and thoroughly mixed for RNA extraction and testing for the levels of aflatoxin.

RNA extraction and sequencing

Total RNA was extracted from 40 mg of finely ground kernel samples using the Spectrum™ Plant Total RNA Kit; Sigma-Aldrich, St. Louis, MO, 2010, according to manufacturer's protocol., except for some samples in lysis solution that needed to be filtered twice. The total RNA was qualified and quantified with an Experion RNA HighSens Analysis Kit; Bio-Rad, Hercules, CA. Approximately 1.4 µg of total RNA was used for cDNA synthesis, followed by construction of RNA-Seq libraries using the TruSeq RNA kit version 2.0, Illumina (San Diego, CA). Samples were submitted for sequencing at BGI Americas (Cambridge, MA) using a module of 100PE (paired ends) on the Illumina HiSeq 2000 platform. The clean reads were sorted according to the barcode of its library and extracted using the BGI pipeline.

These clean reads were deposited at https://www.ncbi.nlm.nih.gov/sra, with Project Number PRJNA384648.

Transcriptome assembly and quality assessment

A *de novo* assembly of transcripts and genes were made at the High Performance Research Computing resources at Texas A&M University through application of the Trinity platform (Haas et al. 2013) on the 24 samples to form the basis of a single Trinity.fasta file. Twenty-four RSEM gene and isoform results files were generated from the Trinity.fasta file in conjunction with the 48 left and right compressed fastq files. Trinity functions were run to compare biological replicates and determine the relatedness of samples. After preliminary analysis, a decision was made to eliminate one silk channel sample and one non-inoculated sample from the milk group in the differential expression analysis that were clear outliers as shown in the PCA (Figure 1) and other graphic comparisons of replicates. The blister stage consisted only of the three treatments without replicates, there were three replicates at the milk stage, and two replicates each at the dough and dent stages were run as depicted in Table 13. Because the mRNA from a fungus-inoculated sample was a mixture of mRNA's from the host plant and pathogen, fungal sequences were identified in the Trinity.fasta file through the application of BLAST+ against the downloaded cDNA file of *Aspergillus flavus* (NRRL3357) which was obtained from the website: http://fungi.ensembl.org/info/website/ftp/index.html. A maximum p-value for identifying *A. flavus* cDNA sequences was set to 1e -20. These fungal sequences were characterized in BLASTn and summarized in Appendix 9, and their Trinity ids were used as a filter to remove them from the maize count matrices.

Statistical models and differential gene expression

Differential gene expression analysis was conducted using edgeR from Bioconductor (Robinson, McCarthy, and Smyth 2010), and run independently of Trinity for greater flexibility in statistical modeling. This R package references a table of actual (or expected) read counts with columns corresponding to the sample libraries, and rows corresponding to the assembled transcripts. The application of a negative binomial distribution in this package assumes that the true gene abundances follow a gamma distribution across replicate samples. A series of functions in R were designed to call upon edgeR routines to: 1) filter out lowly expressed genes with read counts less than 5; 2) implement the experimental design which consisted of two main types of contrasts for differential gene expression: one maturity level versus another, and one inoculation method versus non-inoculated, at each maturity level; 3) calculate and apply trimmed mean of M-values (TMM) normalization scaling factors to correct for the differences in library sizes (Dillies et al. 2013); 4) estimate gene-specific dispersion appropriate for the negative binomial model to account for the biological coefficients of variation expected to exist among genes; 5) conduct a likelihood ratio test (LRT) to determine significant differential gene expression defined by $p \leq .05$, FDR $\leq .10$ and log2FC ≥ 2; and finally 6) Run a modified " TopTags" in edgeR to find the "n" most significant DEGs, in which the BH method is applied to control the false discovery rate (Benjamini and Hochberg 1995). In 4), the gene-specific dispersion factor is calculated on the entire set of samples, at first as a common factor to all genes in all samples, followed by a tagwise dispersion. This provides some correction to gene expression measured for treatments that are lacking replicates, as was the case with the blister group.

Differences in read counts between inoculation methods

A paired t-test was applied to average numbers of reads among replicates for each gene between samples inoculated by silk channel with those inoculated by side needle at each level of maturity, using a similar statement in R: t.test(blist_side, blist_silk, paired = TRUE).

Identification of differentially expressed genes

The primary database referenced for identification of each significant differentially expressed gene as represented by a Trinity.fasta sequence was MaizeGDB (Lawrence et al. 2008) that provided Gramene numbers based on v4 of the maize B73 reference genome. The database most commonly accessed within MaizeGDB was MaizeCyc (Monaco et al. 2013), that provided the name of the most likely gene product, but often Pfam (Finn et al. 2013) and InterPro (Hunter et al. 2011), (Apweiler et al. 2014) were checked as well. NCBI BLASTn (Johnson et al. 2008) was consulted for sequences with alternative characterizations. Original articles were also referenced for gene identities, function and biological processes, especially with respect to pathogenic responses and disease resistance.

Measurement of aflatoxin contamination

Sub-samples of kernels ground for each treatment ranging from 16 to 35 g per ear were tested for aflatoxin concentration using the VICAM AflaTest®per manufacturer's instructions and as used and described in more detail in Wahl et al. (2017).

4. CONCLUSION

The search for the genetic key to control or eliminate aflatoxin contamination of pre-harvest maize is one that has, by necessity, led down more than one path. The most significant factors associated with this highly quantitative trait discussed in this thesis include physical barriers such as good husk coverage, flinty endosperm, hybrid vigor, and in this study, those coding for chitinases, ascorbate peroxidase, heat shock proteins, certain lipoxygenases, genes associated with the production of lignin and that of jasmonic acid, although these are just a small percentage of those reported as inhibitory. Testing for the right combination of at least some of these traits in hundreds of hybrids in different environments over more than a decade has resulted in about one-half of the hybrids, especially those developed by the USDA/ARS in Mississippi, that are superior to checks and other test hybrids in resistance to aflatoxin production, but often were lacking in competitive yield and other desirable agronomic traits. However, thirteen hybrids were identified in the SERAT study that already exhibit both competitive yields, and lower aflatoxin levels compared to checks, and some have already been selected for additional testing.

The necessity to elucidate resistance mechanisms at the genomic and transcriptomic levels in order that elite germplasm might be enhanced without sacrificing yield has encouraged investigators to conduct quantitative trait mapping studies, multiple genome-wide associated studies on large mapping panels, and differential gene and/or protein expression studies to get a closer look at what makes certain maize inbreds or hybrids more

resistant than others. All of this information, across environments, is important to ultimately transfer these traits to high-performing germplasm.

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APPENDIX A

SERAT

Appendix 1. Raw data on yield and agronomic traits of all hybrids

Location	Year	Yield t/ha	Std. error	Min	Max	Plant ht. cm	Ear ht. cm	Stem Ldging %	Root Ldging %	Days to silking	Days to anthesis
College Station, TX	**2006**	7.62	0.27	2.10	13.30	-					
	2007	11.02	0.26	3.30	15.60	244.0	95.0	3.1	5.2	78	
	2008	8.13	0.18	3.90	12.10	252.1	99.8			79	
	2009	9.70	0.17	4.20	14.40	239.7	91.3	8.2	2.2	83	78
	2010	6.26	0.22	0.50	10.80	237.9	89.0			84	82
	2011	8.52	0.16	4.60	10.90	217.6	76.6			67	65
	2012	12.09	0.25	5.70	17.70	261.9	94.4			60	59
	2013	7.34	0.15	4.30	11.50	237.7	102.3			75	74
	2014	10.71	0.18	5.60	14.70	249.3	89.6			74	72
	2015	8.67	0.19	3.49	14.15	256.8	96.6			62	61
College Station Average		8.98	0.09			244.9	93.3			**74**	**73**
Tifton, GA	**2006**	10.57	0.21	4.00	15.40	199.7	100.6	10.2	7.0	64	63
	2007	12.66	0.22	7.10	16.90	215.9	116.9	2.2	0.4		
	2008	9.20	0.32	2.20	15.50	243.3	124.5	3.3	2.0		64
	2009	8.93	0.22	3.30	13.10	257.0	92.6	0.3	2.4		
	2010	9.47	0.26	3.40	14.80	277.0	118.8	0.7	3.0		
	2011	11.86	0.24	6.80	17.60	252.7	106.8	0.4	0.0	56	
	2012	9.19	0.22	4.70	16.80	270.4	115.3	9.5	3.3	61	
	2013	10.60	0.28	2.60	15.00	260.1	108.9	6.5	7.3	66	
	2014	7.02	0.24	1.00	12.70	223.8	82.1	1.8	0.3	64	
	2015	9.53	0.25	1.45	14.58	246.9	96.9	4.6	24.2	57	
Tifton Average		9.80	0.09			241.4	104.7	5.5	6.6	**62**	**64**

Appendix 1. Continued

Location	Year	Yield t/ha	Std. error	Min	Max	Plant ht. cm	Ear ht. cm	Stem Ldging %	Root Ldging %	Days to silking	Days to anthesis
Starkville, MS	**2007**	8.26	0.16	4.30	11.10						
	2011	3.65	0.13	1.40	6.60					59	
	2012	6.09	0.12	2.40	8.60					70	
	2013	7.15	0.16	1.20	10.60					57	
	2014	9.26	0.15	3.70	13.10					64	
	2015	7.92	0.16	4.11	11.85					59	
Starkville Average		7.39	0.08							**62**	
Lubbock, TX	**2007**	10.27	0.28	3.80	16.90	282.6	114.8	3.7	0.0	76	
	2008	8.20	0.23	1.80	12.50	236.5	86.9	2.3	0.0	79	
	2009	9.03	0.23	3.60	14.20	288.7	129.5	1.2	0.0	76	
	2010	12.04	0.26	4.30	17.90	277.5	120.0	2.5	0.0	68	
	2011	5.05	0.21	0.20	10.30	218.4	84.4	0.0	0.0	77	
	2014	9.06	0.17	3.50	13.10	228.0	102.3	0.3	0.0	73	
Lubbock Average		8.94	0.12			254.4	106.4	2.4	**0.0**	75	
Ganado, TX	**2009**	4.20	0.12	1.20	6.20						
	2010	6.80	0.18	2.60	10.30		5.9	28.3			
Ganado Average		5.57	0.19								
Kinston, NC	**2008**	5.40	0.20	1.30	8.70	260.7	104.7	5.4	4.8		
	2011	6.33	0.16	3.80	9.50	253.1	106.3	0.0	27.6		
	2012	6.13	0.33	2.60	8.30	244.6	98.7	0.0	68.6		
	2013	8.03	0.15	3.50	10.80	259.2	103.9	0.8	0.0		
	2015	6.75	0.15	2.26	9.54	289.8	119.5	1.0	0.2		
Kinston Average		6.56	0.09			263.63	107.35	1.81	1.07		
Lewiston, NC	**2008**	5.59	0.22	1.90	8.60	232.4	80.7	5.4	0.9		
	2009	7.46	0.16	3.20	10.10	259.2	124.5	1.1	0.8		
	2010	4.59	0.16	1.50	7.10	257.5	113.6	1.1	0.1		
	2011	6.38	0.19	3.60	8.80	240.4	90.7	0.0	0.0		
	2012	7.53	0.23	3.10	11.70	266.8	102.5	0.1	0.0		
	2013	8.49	0.18	1.40	11.40	253.1	97.0	0.4	0.0		
	2015	8.50	0.16	5.21	11.30	289.1	125.8	2.3	0.0		
Lewiston Average		7.02	0.09			258.24	106.21	1.98	0.44		

Appendix 2. Levels of aflatoxin contamination of all hybrids

Location	Year	Aflatoxin ng g^{-1}	Min	Max	Log_{10}(afl + 1)	Aflatoxin † GM ng g^{-1}	L 95 CI	U 95 CI
College Station, TX	2006	229	3	2100	2.08	118	92	152
	2007	175	0	1100	1.89	77	55	107
	2008	162	3	900	1.99	98	77	124
	2009	136	0	1400	1.87	74	58	94
	2010	312	0	1300	2.24	172	133	223
	2011	786	230	2500	2.83	680	597	775
	2012	111	0	1300	1.68	47	33	66
	2013	154	4	1100	1.94	85	69	106
	2014	131	0	950	1.86	72	57	91
	2015	216	0	1500	1.97	93	70	125
College Station Average		227	0	2500	2.02	103	94	113
Tifton, GA	2006	546	60	4900	2.53	334	286	390
	2007	755	120	6100	2.70	506	435	**588**
	2008	354	25	2200	2.40	253	219	291
	2009	284	47	1000	2.32	206	157	270
	2011	181	21	710	2.15	141	122	163
	2012	217	17	2500	2.13	135	115	158
	2013	213	17	1700	2.16	145	126	167
	2014	375	18	4600	2.38	237	207	272
	2015	121	7	980	1.97	93	83	104
Tifton Average		337	7	6100	2.30	197	186	209
Starkville, MS	2007	631	8	6000	2.55	352	272	457
	2008	1278	14	8888	2.83	670	522	860
	2009	265	0	1480	2.02	104	74	144

Appendix 2. Continued

Location	Year	Aflatoxin ng g^{-1}	Min	Max	Log_{10}(afl + 1)	Aflatoxin † GM ng g^{-1}	L 95 CI	U 95 CI
	2010	199	0	960	1.97	92	69	122
	2011	566	5	4800	2.48	299	229	390
	2012	677	0	11200	2.31	204	143	290
	2013	202	0	1800	1.91	81	60	108
	2014	155	0	1080	1.82	66	49	88
	2015	218	0	1440	1.95	87	64	118
Starkville Average		450	0	11200	2.18	150	135	167
Lubbock, TX	2011	241	5	630	2.23	169	138	208
	2012	113	43	203	2.02	105	169	138
	2014	39	3	180	1.48	29	105	90
Lubbock Average		123	3	630	1.82	66	57	76
Average over all environments †		323			2.15	139	132	146
Average check - all environments †		370			2.27	187	169	207
Average program - all environments †		313			2.12	130	123	138

† Values based upon log(aflatoxin +1) or back-transformed geometric means

Appendix 3. Genotypic and phenotypic correlations among traits by environment for all hybrids

		Yld & Plant Ht		Yld & Ear Ht		Plt Ht & Ear Ht	
Location	**Year**	**Phen†**	**Gen±**	**Phen**	**Gen**	**Phen**	**Gen**
College Station, TX	**2006**	-					
	2007	0.13	0.10	0.09	0.10	0.54***	0.78***
	2008	0.20	-0.5**	0.20	-0.4*	0.74***	0.75***
	2009	-0.09	-0.1	-0.15	-0.41*	0.82***	0.82***
	2010	0.24*	0.06	-0.02	-0.24	0.88***	0.91***
	2011	0.19	0.42*	-0.22	-0.08	0.41***	0.65***
	2012	0.71***	0.77***	0.39***	0.4*	0.63***	0.7***
	2013	0.21*	0.43**	0.08	0.21	0.65***	0.74***
	2014	0.03	-0.07	-0.24**	-0.37*	0.68***	0.78***
	2015	-0.11	-0.19	-0.31***	-0.43**	0.81***	0.89***
Tifton, GA	**2006**	0.28***	0.09	0	-0.30	0.69***	0.72***
	2007	0.25*	0.46*	0.32**	0.39*	0.7***	0.83***
	2008	0.27*	0.26	0.05	-0.02	0.76***	0.88***
	2009	0.14	0.08	-0.31**	-0.52**	0.4***	0.64***
	2010	-0.07	-0.19	-0.32**	-0.49**	0.76***	0.87***
	2011	0.45***	0.68***	0.33**	0.31	0.65***	0.74***
	2012	0.21*	0.28	-0.13	-0.14	0.68***	0.71***
	2013	0.3*	0.49**	0.41***	0.38*	0.33**	0.51**
	2014	0.11	0.01	0.05	-0.12	0.75***	0.8***
	2015	-0.15	-0.22	-0.36***	-0.48**	0.79***	0.87***

Appendix 3. Continued

Location	Year	Yld & Plant Ht Phen†	Yld & Ear Ht Gen±	Plt Ht & Ear Ht Phen	Plt Ht & Ear Ht Gen	Phen	Gen
Lubbock, TX*	**2007**	0.09	0.11	-0.09	-0.10	0.74***	0.86***
*** DTA**	**2008**	-0.07	0.00	-0.11	-0.14	0.81***	0.89***
	2009	0.12	-0.08	-0.52***	-0.63***	0.5***	0.69***
	2010	-0.15	-0.21	-0.31**	-0.44*	0.88***	0.91***
	2011	0.13	0.20	0.15	0.16	0.75***	0.9***
	2014	-0.32***	-0.39*	-0.41***	-0.54***	0.73***	0.82***
Kinston, NC	2008	0.08	-0.07	-0.25*	-0.19	0.78***	0.08
	2011	0.61***	-0.16	0.2	0.28	0.63***	0.06
	2012	0.49***	0.58**	0.27*	0.19	0.69***	0.77***
	2013	0.59***	0.20	0.43***	0.41*	0.7***	0.24
	2015	0.10	-0.08	-0.18	-0.35*	0.81***	0.91***
Lewiston, NC	2008	-0.3*	0.08	-0.47***	-0.17	0.95***	0.93***
	2009	0.22	0.11	-0.12	-0.36*	0.7***	0.78***
	2010	-0.13	-0.26	-0.53***	-0.61***	0.75***	0.84***
	2011	0.65***	0.7***	0.56***	0.39*	0.65***	0.76***
	2012	0.53***	0.59***	0.22	0.19	0.73***	0.76***
	2013	0.46***	0.6***	0.4***	0.47**	0.73***	0.75***
	2015	-0.25*	-0.26	-0.32**	-0.42**	0.89***	0.94***
Clayton, NC	2009	0.07	-0.02	-0.35**	-0.51**	0.7***	0.76***

Appendix 3. Continued

		Yld & DTS		Yld & Ldg		Yld & Log(AFL + 1)		DTS & Log(AFL + 1)	
Location	**Year**	**Phen**	**Gen**	**Phen SL%**	**Phen RL%**	**Phen**	**Gen**	**Phen**	**Gen**
College Station, TX	**2006**					-0.32***	-0.38*		
	2007	0.11	0.21	-0.55***	-0.45***	-0.03	-0.01	0.03	0.14
	2008	0.42***	0.51**			0.11	0.38*	0.26*	0.47**
	2009	-0.51***	-0.69***	-0.47***	-0.44***	0.45***	0.64***	-0.12	-0.43*
	2010	-0.06	-0.19			0.04	0.2	-0.33***	-0.65***
	2011	-0.13	-0.15			-0.12	0.03	-0.08	-0.26
	2012	-0.01	0.01			0.06	0.02	-0.12	-0.13
	2013	-0.15	-0.12			-0.13	-0.23	-0.37***	-0.46**
	2014	-0.2*	-0.28			0.29**	0.42**	-0.13	-0.26
	2015	-0.38***	-0.45**			0.14	0.25	-0.04	-0.18
Tifton, GA	**2006**	-0.31***	-0.25	-0.64***	-0.22**			-0.07	-0.29
	2007			-0.46***	-0.2				
	2008			-0.55***	-0.01				
	2009			-0.16	-0.48***				
	2010			-0.16	-0.51***				
	2011			-0.5***	0.00			-0.31**	-0.44*
	2012			-0.53***	-0.25*			-0.57***	-0.66***
	2013			-0.54***	-0.51***			-0.22**	-0.35*
	2014			-0.55***	-0.15			-0.29***	-0.4**
	2015			-0.4***	-0.71***			0.00	-0.20
Starkville, MS	**2007**					-0.27*	-0.22		

Appendix 3.
Continued

		Yld & DTS		Yld & Ldg		Yld & Log(AFL + 1)	DTS & Log(AFL + 1)		
Location	**Year**	**Phen**	**Gen**	**Phen SL%**	**Phen RL%**	**Phen**	**Gen**	**Phen**	**Gen**
	2010					0.2*	0.44**		
	2011	-0.31**	-0.43*			0.1	0.27	-0.26*	-0.45*
	2012	-0.18*	-0.23			-0.2*	-0.06	-0.43***	-0.51***
	2013	-0.17	-0.17			-0.07	-0.14	-0.62***	-0.71***
	2014	-0.16	-0.23			0.23*	0.37*	-0.36***	-0.44**
	2015	-0.48***	-0.53***			0.25**	0.33*	-0.51***	-0.54***
Lubbock, TX*	**2007**	0.04	0.15	-0.49***	0.00				
*** DTA**	**2008**	-0.32***	-0.36*	-0.42***	0.00				
	2009	-0.55***	-0.64***	-0.38***	-0.13				
	2010	-0.07	-0.15	-0.17	0.00				
	2011	-0.35***	-0.22	0.14	0.00	-0.13	-0.03	-0.04	-0.12
	2014	-0.32***	-0.4**	-0.17	-0.1	0.08	0.30	-0.21*	-0.39*
Ganado, TX	**2009**								
	2010			-0.35***	-0.46***				

Appendix 3. Continued

		Yld & Ldg	
	Year	**Phen SL%**	**Phen RL%**
Kinston, NC	**2008**	0.15	-0.44***
	2011	0.00	0.00
	2012	0.00	0.00
	2013	-0.24*	-0.1
	2015	-0.15	0.04
Lewiston, NC	**2008**	-0.36**	-0.35**
	2009	-0.18	-0.51***
	2010	-0.39**	-0.33**
	2011	0.00	0.00
	2012	-0.23	0.00
	2013	-0.38***	0.00
	2015	-0.29**	-0.14
Clayton, NC	**2009**	-0.26*	-0.37**

†Phen refers to correlations determined on raw, phenotypic measurements.
‡Gen refers to correlations determined on BLUPs.

Appendix 4. Measures of yield stability by year		Yield		
Pedigree	**Year**	**BLUPs**	**Slope**	**MSE**
Mo18W x Mp313E	2008	6.45	1.36	0.65
W07-038/LH287	2008	9.01	1.27	0.26
Mp04:97 x Mp313E	2008	5.57	1.22	0.90
GA209 x SC212M	2008	6.20	1.19	0.03
Y07-095/LH195	2008	8.09	1.18	0.62
P31P41	2008	10.28	1.17	0.59
DW997FL x LH287BT1CCR1	2008	8.93	1.16	0.59
DW909FL x LH287BT1CCR1	2008	8.94	1.12	0.17
NC300 x S2B73BC	2008	8.72	1.12	0.33
B110 x BR-1	2008	9.19	1.06	0.10
FR1064 x LH287BT1CCR1	2008	8.54	1.06	0.11
Y07-118/LH195	2008	8.21	1.06	0.38
GT602 x AT805	2008	6.03	1.05	1.78
DW893FL x LH287BT1CCR1	2008	8.94	1.04	0.06
Tx204 x CML32xB104)F7-2-1-b-1-B-2-1-	2008	8.46	1.04	0.35
DW933FL x LH287BT1CCR1	2008	9.18	1.02	0.25
B73 x GTP50	2008	7.28	1.01	0.17
DW1022FL x LH287BT1CCR1	2008	9.04	0.99	0.27
Mp04:97 x Mp07:117	2008	5.89	0.98	0.28
Mp04:97 x Mo17	2008	6.64	0.96	0.08
CML273xA632)F7-1b-1-1-B x Tx205	2008	7.80	0.94	0.16
C3A654-3-2-1-1-1-1-1-1 x LH195Bt1RR2-1	2008	7.44	0.91	0.30
AT805 x GT602	2008	7.42	0.90	0.23
C3S1B73-1-1-1-1-B-1-1-B x LH287BT1RR2-1	2008	7.81	0.89	0.19
Mp04:97 x B73	2008	6.95	0.88	0.18
Mp07:117 x Mp313E	2008	5.43	0.88	1.35

Appendix 4. Continued

Pedigree	Year	Yield BLUPs	Slope	MSE
Y07-111/LH195	2008	8.23	0.86	0.35
FR1064 x FR6942HX1.1	2008	7.67	0.86	0.57
DK697	2008	9.54	0.85	0.88
AT805 x P50	2008	7.36	0.85	0.04
P31D58	2008	9.17	0.82	1.55
P50 x AT805	2008	3.55	0.82	0.26
B110xCML343xS1)XB73)F5xMP715-1-4-7-B-1-1-1-1 x C2A554-4-2-1-B-1	2008	7.80	0.78	0.68
Y07-055/LH195	2008	8.20	0.77	0.28
Mo17 x GTP50	2008	2.60	0.67	0.05
Mp 04:97 x Mp 04:110	2009	5.67	1.18	1.57
GT P50 x Mo17	2009	8.23	1.17	0.40
S2B73 x NS	2009	9.77	1.16	0.30
Mp04:107 x LH310	2009	7.58	1.15	0.53
DK697	2009	10.67	1.14	1.08
C2A632 x NS	2009	9.45	1.14	0.16
P31P41	2009	10.58	1.09	0.80
GT 601 x DK 888	2009	8.38	1.09	0.64
Y07-094/LH195	2009	8.02	1.07	0.47
Mp 313E x GT 601	2009	8.37	1.06	0.83
DW893FL x LH287BT1CCR1	2009	8.98	1.04	0.36
BMP-1-4-7 x C2A554-4	2009	8.71	1.04	0.13
DW933FL x LH287BT1CCR2	2009	8.86	1.02	0.25
Mp04:107 x LH195	2009	8.02	1.01	0.08
Y07-131/Y07-095	2009	7.68	1.00	0.23

Appendix 4. Continued

Pedigree	Year	Yield BLUPs	Slope	MSE
P31D58	2009	9.92	0.99	0.37
CUBA1 x NS	2009	9.49	0.99	0.51
DW893FL x TAMU 2 ((CML288/NC300)-B-9-B1-B-B-B-B))	2009	7.78	0.98	0.63
Mp313E x Mp04:97	2009	5.76	0.98	0.44
Mp04:115 x LH195	2009	8.50	0.97	0.11
CUBA1 x BR1	2009	8.47	0.96	0.03
DW1064 x LH287BT1CCR4	2009	9.52	0.95	0.21
Y07-114/LH287	2009	9.22	0.95	0.27
B5C2 x NC300	2009	8.58	0.95	0.46
GT 601 x AT 709	2009	8.52	0.95	0.29
Y07-164/LH195	2009	8.17	0.95	0.19
DW997FL x LH287BT1CCR3	2009	9.12	0.94	0.14
Mp05:115 x LH310	2009	8.64	0.93	0.18
Mp04:97 x LH310	2009	8.22	0.90	0.36
DW997FL x TAMU 4 ((Tx601 x Tx772)-B-B-20-1-1-B-B-B-B))	2009	7.70	0.88	0.14
DK888 x GT 601	2009	8.37	0.86	0.93
AT709xGT601	2009	8.58	0.81	0.42
(LH195RR2.1xMP313E)BC7P1S1 x LH210	2009	8.86	0.80	0.33
GT P50 x B73	2009	9.07	0.78	0.18
DK697	2010	9.69	1.25	0.33
Syn AM 1 (P43) x GP 282	2010	7.59	1.23	1.03
Mp313E x Mp317	2010	7.32	1.15	0.08
WE09-ISO-Prp-64-Yel	2010	7.13	1.12	0.17

Appendix 4. Continued		**Yield**		
Pedigree	**Year**	**BLUPs**	**Slope**	**MSE**
Cy-2 x LH132 .FR1064	2010	8.80	1.11	0.08
CUBA1 x NS	2010	9.28	1.10	**0.12**
WE06-6001-TAC-Yel	2010	7.28	1.08	0.18
DK-7 x SS	2010	9.62	1.07	0.09
CS09-QPMX-059-Wh	2010	7.03	1.07	0.39
PRA96A x NS	2010	8.85	1.04	0.32
CS08-TAC - Yel	2010	7.39	1.04	0.33
AT709xGT601	2010	7.35	1.04	0.25
LB08Iso:8039	2010	7.91	1.03	0.14
P31P41	2010	9.80	1.02	0.53
P31D58	2010	9.42	1.02	0.17
H08:106x139	2010	7.58	1.02	0.26
CS09-QPMX-050-Wh	2010	6.80	1.01	0.77
Mp313E x Mo18W	2010	6.45	1.01	0.37
GT P50 x DK888 N11 F1s3 2141-2-34-B-2-1	2010	7.49	0.98	0.28
GP282 x GT P50	2010	7.51	0.96	0.69
WE09-ISO-Pro-111-Yel	2010	8.32	0.95	0.17
Syn AM 1 (P43) x GP 280	2010	7.10	0.95	0.48
C2A632-1a x NS	2010	8.67	0.91	0.23
CS09-QPMX-005-Blue	2010	6.47	0.88	0.11
CY-5 x LH132.FR1064	2010	6.02	0.88	0.10
Mp494 x Mp717	2010	5.48	0.87	0.19
Mp313E x Mp715	2010	7.04	0.86	0.49
Mp715 x Mp717	2010	4.86	0.82	0.47
S2B73BC x NS	2010	9.07	0.79	0.44

Appendix 4. Continued

Pedigree	Year	Yield BLUP	Slope	MSE
NC300 x Mp715	2010	4.12	0.65	0.55
P31P41	2011	8.60	1.13	0.05
DK697	2011	8.32	1.13	0.01
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B2/SS	2011	6.84	1.11	0.24
AT709xGT601	2011	6.11	1.09	0.39
P31G98	2011	8.44	1.08	0.02
CUBA1 x NS2	2011	7.02	1.08	0.20
GP282 X GT603	2011	6.25	1.07	0.26
CUBA1 x NS	2011	7.42	1.06	0.18
DK-7 x SS	2011	7.83	1.05	0.28
S2B73BC x NS	2011	8.59	1.04	0.42
ArgnetineFlintyComposite-C(1)-14-B-B/SS	2011	6.82	1.02	0.12
CUBA1 x NS3	2011	7.63	1.01	0.18
GT603 x DK888N11Fls3,2141-2-34-B-2-1	2011	5.35	0.99	0.28
C2A632 x NS	2011	7.85	0.98	0.53
BMP-14-7 x A2A554-4	2011	7.04	0.98	0.43
((CML288/NC300)-B-9-B1-B-B-B-B-B-B-B)xLH132	2011	5.55	0.98	0.82
ArgnetineFlintyComposite-C(1)-15-B1-B/SS	2011	6.52	0.95	0.06
PR96A x NS	2011	8.02	0.94	0.25
BR-1 x SS	2011	7.11	0.94	0.01
Lo964 x GT603	2011	5.42	0.94	0.28
CY1 x NC262B	2011	6.78	0.92	0.01
((LAMA2002-2-5-B/(CML285/B104)-B-4-B-B-B-B)-B-B2-2-3-B-B)xLH132	2011	5.78	0.87	0.17
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1/NSS	2011	5.91	0.85	0.45
((LAMA2002-12-1-B/(CML 325/B104)-B-1-B-B-B-B)-B-B2-3-2-B-B)xLH132	2011	6.20	0.84	0.31

Appendix 4. Continued

Pedigree	Year	Yield BLUP	Slope	MSE
P31P41	2012	10.64	1.25	0.66
CUBA1 x NS3	2011	7.63	1.01	0.18
GT603 x DK888N11Fls3,2141-2-34-B-2-1	2011	5.35	0.99	0.28
C2A632 x NS	2011	7.85	0.98	0.53
BMP-14-7 x A2A554-4	2011	7.04	0.98	0.43
((CML288/NC300)-B-9-B1-B-B-B-B-B-B-B)xLH132	2011	5.55	0.98	0.82
ArgnetineFlintyComposite-C(1)-15-B1-B/SS	2011	6.52	0.95	0.06
PR96A x NS	2011	8.02	0.94	0.25
BR-1 x SS	2011	7.11	0.94	0.01
Lo964 x GT603	2011	5.42	0.94	0.28
CY1 x NC262B	2011	6.78	0.92	0.01
((LAMA2002-2-5-B/(CML285/B104)-B-4-B-B-B-B)-B-B2-2-3-B-B)xLH132	2011	5.78	0.87	0.17
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1/NSS	2011	5.91	0.85	0.45
((LAMA2002-12-1-B/(CML 325/B104)-B-1-B-B-B-B)-B-B2-3-2-B-B)xLH132	2011	6.20	0.84	0.31
P31P41	2012	10.64	1.25	0.66
BH8910RR/HX	2012	9.97	1.17	1.11
BH9051RR	2012	9.22	1.16	0.01
BH8740VTTP	2012	9.54	1.14	0.36
Tx-WX12-01	2012	9.30	1.13	0.09
((Tx741) ; LAMA2002-42-B-B-B-B-B3) X SS3	2012	8.48	1.11	0.16
LH132 x GTA2R	2012	7.40	1.08	0.01
Tx-WX12-02	2012	9.09	1.06	0.08
P31G98	2012	10.16	1.05	0.13
Hi63xNC466	2013	7.33	0.90	0.24
GEMS 0005-2-1B X Hi27bs	2013	7.54	0.87	0.04

Appendix 4. Continued

Pedigree	Year	Yield BLUP	Slope	MSE
BH8910RR/HX	2013	9.71	0.84	0.36
GTA2R-1B-1B X SC212M	2013	6.33	0.84	2.87
GTA2R-1B-1B X TUN 85	2013	7.04	0.80	0.31
HBA1-1-1-1B X GT-603	2013	7.30	0.79	0.15
(CML288/NC300)-B-9-B1-B-B-B-B-B-B-B-B14 X LH195 (GRIN-PI)	2013	7.95	0.70	0.27
TUN18-2 x GT603	2013	6.04	0.61	0.35
CUBA1TEO21 x NS	2013	4.15	0.37	0.35
P2088R	2014	11.29	1.50	0.45
P1745R	2014	11.52	1.35	0.33
B5C2RM-45-1-1 x NS1	2014	8.15	1.29	1.44
DK68-04	2014	9.15	1.27	0.33
P31P41	2014	11.16	1.25	0.48
Tx777 X SS3	2014	10.87	1.19	0.49
BH8740VTTP	2014	10.02	1.17	0.66
SS1 x C2A5-4	2014	9.41	1.17	0.95
GT A2 R 1B 1B x DK888	2014	8.48	1.17	0.94
SS1 x C2A5-2	2014	9.14	1.13	1.12
DK7 x SS1	2014	12.34	1.12	0.21
BH8910RR/HX	2014	11.27	1.12	0.21
P31G98	2014	10.92	1.12	1.30
SS1 x Tx207	2014	8.48	1.10	0.50
Tx777\X\LH195	2014	11.49	1.09	0.27
SS1 x C2A5-3	2014	8.41	1.06	1.16
SS2\X\(CML450-B/Tx110)-B-3-B-1-B-B-1-1-B18	2014	10.91	1.00	0.34
SS1 x C2A5-1	2014	9.89	1.00	0.32

Appendix 4. Continued

Pedigree	Year	Yield BLUP	Slope	MSE
SS1 x Tx208	2014	9.63	1.00	0.20
PHG39 x DK888	2014	8.97	0.97	0.24
LAMA2002-58-3-B-B-B-B-B-B-1-B19\X\NSS1	2014	10.59	0.96	0.21
BR-1 x SS1	2014	10.34	0.96	0.21
DK697	2014	11.00	0.95	0.14
GEMS-0028-2-1 x GT603	2014	8.41	0.94	0.32
(LAMA2002-22-1-B-B-B-B/LAMA2002-1-5-B-B-B-B)-2-1-B-1-1-1-B19-B18\X\LH195	2014	9.98	0.92	0.86
GRACE E-5 (E-1) x DK888	2014	8.89	0.91	0.26
Hi63xNC466	2014	8.55	0.91	0.07
SS1\X\(LAMA2002-10-1-B/(CML288/NC300)-B-9-B1-B-B-B)-B-B-1-3-B-1-B	2014	10.43	0.89	0.26
(LAMA2002-23-1-B-B/LAMA2002-11-1-B-B)-B-B-B-B-B-1-B6\X\SS1	2014	9.53	0.85	0.04
((Tx740/Mp715)//(Tx772/Mp313))-#/((Tx772/Mp715)//(Tx740/Mp313E))-#	2014	6.18	0.85	0.28
Mp13:9011 x Mp13:9012	2014	8.20	0.84	0.11
BH9051RR	2014	10.01	0.83	0.05
SS1\X\(LAMA2002-35-2-B-B-B-B/CG44)-1-3-B-B14-B10	2014	9.85	0.83	0.70
Mp13:9025 x Mp13:9026	2014	7.59	0.82	0.51
SYN AM P43 x DK888	2014	8.78	0.78	0.12
Hi31 x GT603	2014	7.27	0.72	0.65
FAW 1430 x NC358	2014	9.09	0.69	0.51
Mp13:9035 x Mp13:9036	2014	7.63	0.69	1.11
Terral 28R20	2015	12.36	1.52	0.26
GP474GT/Tx777	2015	10.80	1.40	0.36
P31G98	2015	10.79	1.36	0.80
SGI890/Tx777	2015	9.75	1.36	0.06
CUBA1 x NS1	2015	9.79	1.35	0.09

Appendix 4. Continued

Pedigree	Year	Yield BLUP	Slope	MSE
P31P41	2015	10.71	1.33	0.03
P2088R	2015	11.58	1.31	0.13
P1745R	2015	11.57	1.31	0.33
CUBA1TEO33 x NS1	2015	8.75	1.25	0.11
GP286/Tx777	2015	10.82	1.24	0.30
CUBA1TEO30 x NS1	2015	9.00	1.23	0.37
Zm 521 E-1 X B73	2015	9.06	1.19	0.39
GTA1R TP Yellow E-1 X B73	2015	8.68	1.12	0.12
8waf BULK2	2015	7.83	1.11	0.16
ANTIGO2 x SS1	2015	8.77	1.10	0.02
DK68-04	2015	8.27	1.10	0.08
(NC300 x Tx714-B/B104-1/CML343)-2-1-B-B-B-B-B-B-B-B-1-B25/Tx777	2015	10.02	1.06	0.10
CUBATEO90 x NS1	2015	7.96	1.04	0.54
ANTIGO4 x SS1	2015	9.91	1.03	0.29
DK64-69	2015	10.35	1.00	0.16
ANTIGO6 x SS1	2015	9.35	0.99	0.22
DK697	2015	10.66	0.95	0.15
FAW 1430 x NC358	2015	6.73	0.94	1.00
GP280GT/Tx777	2015	9.71	0.90	0.06
ANTIGO19/20 x SS1	2015	9.20	0.87	0.11
8waf BULK1	2015	7.35	0.85	0.04
CUBA1TEO21 x NS1	2015	5.05	0.82	0.50
LH195 X GT1318	2015	8.52	0.80	0.30
Mp13:9031 x Mp13:9032	2015	7.88	0.79	0.28
LH210 X GT1214	2015	7.71	0.78	0.14
Mp13:9021 x Mp13:9022	2015	5.49	0.78	0.27

Appendix 4. Continued

Pedigree	Year	Yield BLUP	Slope	MSE
NP2643GT/Tx777	2015	9.95	0.76	0.68
GTA1R TP Yellow E-1 X Mo17	2015	7.97	0.75	0.13
8waf BULK3	2015	6.83	0.72	0.05
Mp13:9013 x Mp13:9014	2015	7.31	0.67	0.25
LH51 X Gems0005-2-1	2015	9.21	0.61	0.41
Mp13:9027 x Mp13:9028	2015	6.32	0.59	0.09
Oh43 x FAW 1430	2015	6.99	0.57	0.41
LH210 X GT1309	2015	8.80	0.49	0.31
Mp13:9037 x Mp13:9038	2015	5.86	0.25	0.39

Appendix 5. Comparison of aflatoxin levels and yield in related hybrids

	Mp13	Dataset 2 Log$_{10}$(Afl+1)	Ck avg %	Dataset 1 Yield BLUP	Ck avg %	Slope (b)	
2014	Mp13:9011 x Mp13:9012	1.60	80	8.33	79	0.84	0.11
	Mp13:9025 x Mp13:9026	1.31	65	7.77	74	0.82	0.51
	Mp13:9031 x Mp13:9032†	1.45	72	8.69	82	0.78	0.30
2015	Mp13:9035 x Mp13:9036	1.71	85	7.8	74	0.69	1.11
	Mp13:9031 x Mp13:9032	1.65	76	7.92	75	0.79	0.28
	Mp13:9021 x Mp13:9022	1.56	72	5.63	53	0.78	0.27
	Mp13:9013 x Mp13:9014	1.81	84	7.38	70	0.67	0.25
	Mp13:9027 x Mp13:9028	1.70	79	6.42	61	0.59	0.09
	Mp13:9037 x Mp13:9038	1.59	74	6.63	63	0.25	0.39
	GT603						
2011	GP282 X GT603	2.39	100	6.26	82	1.07	0.26
	GT603 x DK888N11Fls3,2141-2-34-B-2-1	2.33	97	5.48	71	0.99	0.28
	Lo964 x GT603	2.39	99	5.54	72	0.94	0.28
2012	Hi27 x GT603	1.7	82	7.21	74	1.01	0.25
	HBA x GT603	1.93	93	6.94	71	0.98	0.88
	CY1 x GT603	1.93	93	7.34	75	0.90	0.52
2013	GP280 x GT603	2.24	108	8.32	89	1.40	0.25
	GP282 X GT603	1.72	83	7.84	84	1.19	0.19
	TUN18-2 x GT603	2.14	103	6.18	66	0.61	0.35
2014	GEMS-0028-2-1 x GT603	1.45	72	8.53	81	0.94	0.32
	Hi31 x GT603	1.62	80	7.44	71	0.72	0.65
	CUBA1TEO						
2013	CUBA1TEO51-1 x NS	2.27	109	8.49	91	1.08	0.03
	CUBA1TEO67 x NS	2.47	119	8.20	88	1.08	0.22
	CUBA1TEO42 x NS	2.23	108	8.47	91	1.02	0.13
	CUBA1TEO43 x NS	2.28	110	8.72	93	1.01	0.34
	CUBA1TEO41 x NS	2.31	111	8.36	89	0.95	0.49
	CUBA1TEO62 xNS	2.19	106	8.41	90	0.94	0.07
	CUBA1TEO30 x NS	2.16	104	8.39	90	0.90	0.07
	CUBA1TEO21 x NS	2.85	137	4.38	47	0.37	0.35
2015	CUBA1TEO33 x NS1	1.73	80	8.76	83	1.25	0.11
	CUBA1TEO30 x NS1	2.18	101	8.99	85	1.23	0.37
	CUBATEO90 x NS1	1.96	90	7.99	76	1.04	0.54
	CUBA1TEO21 x NS1	2.49	115	5.21	49	0.82	0.50
	Tx777						
2013	Tx777 X SS3	1.64	79	9.95	106	1.25	0.36
	Tx777 X SS2	1.79	86	10.00	107	0.92	0.53
2014	Tx777 X SS3	1.73	86	10.66	101	1.19	0.49
	Tx777 X LH195	1.67	83	11.33	107	1.09	0.27
2015	GP474GT/Tx777	2.06	95	11.33	108	1.40	0.36
	SGI890/Tx777	1.76	81	9.72	92	1.36	0.06
	GP286/(Tx777	1.83	85	10.75	102	1.24	0.30
	(NC300 x Tx714-B/B104-1/CML343)-2-1-B-B-B-B-B-B-B-B-1-B25/Tx777	1.60	74	9.98	95	1.06	0.10
	GP280GT/Tx777	1.91	88	9.68	92	0.90	0.06
	NP2643GT/Tx777	1.89	87	9.91	94	0.76	0.68

† Shaded rows refer to testing of replicate hybrids in different years

Appendix 6. Top hybrids for yield and low to average aflatoxin levels

Year	Pedigree†	Yield BLUPs‡	Percent Check Ave	Log(Afl) BLUPs	Percent Check Ave	Type II Stability	Type III Stability
		Mg ha-1	%		%	β	δ
2006	(**NC300** x Tx714-B/B104-1/**CML343)**-2-1-B-B-B-B/LH210	12.1 ±1.26	125%	2.17 ±0.28	92%		
	(CML285/NC300)-B-6-B-B-B-B/LH195	11.7 ±0.96	121%	2.18 ±0.27	93%		
	S2B73 x NC300	11.17 ±0.96	116%	2.07 ±0.27	88%		
	S1W x CML343	10.71 ±0.96	111%	2.35 ±0.27	100%		
	DW1037 x FR6942HX	10.57 ±0.96	110%	2.33 ±0.27	99%		
	(Tx601 x B104-B/FR2128-B x Bd)-2-1-B-B-B-B/LH210	10.51 ±0.96	109%	2.13 ±0.27	91%		
	CY-1 x A-2	10.41 ±0.96	108%	2.23 ±0.27	95%		
2007	B110 x **BR52051:N04-1**	11.55 ±1.03	93%	2.15 ±0.29	88%		
	(B97x CML 326-B/Tx770 x A645)-1-5-B-B-B/LH195	11.45 ±1.03	92%	2.26 ±0.29	92%		
	S2B73 x NC300	11.31 ±1.03	91%	2.12 ±0.29	86%		
2008	B110 x BR52051:N04-1	9.21 ±0.9	99%	2.15 ±0.28	83%	1.06	0.10
	NC300 x S2B73BC	8.84 ±0.9	95%	2.28 ±0.27	88%	1.12	0.33
2010	**LB08Iso**:8078	9.52 ±1.2	107%	2.13 ±0.16	100%	1.07	0.09
	H08:361x385	9.2 ±1.2	104%	2.11 ±0.16	100%	1.10	0.12
	LB08Iso:6122-3	8.99 ±1.2	102%	2.14 ±0.16	101%	0.79	0.44
	LB08Iso:6108	8.79 ±1.2	99%	2.12 ±0.16	100%	1.04	0.32
	Cy-2 x LH132 .FR1064	8.7 ±1.2	98%	2.1 ±0.16	99%	1.11	0.08
	LB08Iso:6059	8.62 ±1.2	97%	2.1 ±0.16	99%	0.91	0.23
	Mp317 x 50	8.38 ±1.4	95%	2.11 ±0.16	99%		
	WE09-ISO-Pro-111-Yel	8.29 ±1.2	94%	2.11 ±0.16	99%	0.95	0.17
	Mp317 x 26	8.21 ±1.4	93%	2.1 ±0.16	99%		
	Mp317 x **Mp717**	8.19 ±1.4	92%	2.09 ±0.16	98%		

Appendix 6 continued.

Year	Pedigree†	Yield BLUPs‡ Mg ha-1	Percent Check Ave %	Log(Afl) BLUPs	Percent Check Ave %	Type II Stability β	Type III Stability MSE
	Mp317 x 45	7.95 ±1.4	90%	2.07 ±0.16	98%		
2011	CUBA1 x NS3	7.61 ±1.22	99%	2.2 ±0.18	92%	1.01	0.18
	BR-1 x SS	7.12 ±1.22	93%	2.13 ±0.18	89%	0.94	0.01
	((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B2/SS	6.87 ±1.22	90%	2.32 ±0.18	97%	1.11	0.24
2012	**TZAR106** X LH51	8.9 ±1.36	91%	1.33 ±0.31	64%		
	TZAR106 X LH132	8.8 ±1.36	90%	1.45 ±0.31	70%		
	((Tx741) ; LAMA2002-42-B-B-B-B-B3) X SS3	8.46 ±1.2	87%	1.47 ±0.23	71%	1.11	0.16
	TZAR103 X LH51	8.33 ±1.36	85%	1.36 ±0.31	65%		
	[(**Mp494** X GEMN-013) X (Mp717 X GEMS-0074)]	8.12 ±1.28	83%	1.34 ±0.25	64%		
	Mp494 X GEMN-0130	8.07 ±1.28	83%	1.55 ±0.26	75%		
2013	**TX777** X SS2	10 ±0.75	107%	1.79 ±0.17	86%	0.92	0.53
	TX777 X SS3	9.95 ±0.75	106%	1.64 ±0.17	79%	1.25	0.36
	SS1 X (CML450-B/Tx110)-B-3-B-1-B-B-1-1-B18	9.32 ±0.75	100%	1.82 ±0.17	88%	1.00	0.03
	SS1 X (LAMA2002-61-2-BB/LAMA2002-53-5-BB)-B*5-1-B6-1-B16	9.02 ±0.75	97%	1.61 ±0.17	78%	1.13	0.07
	LAMA2002-58-3-B-B-B-B-B-B-1-B19 X NSS2	8.98 ±0.75	96%	1.75 ±0.17	84%	1.12	0.08
	Mp 313E x NC 322	8.85 ±1.00	95%	1.49 ±0.25	72%		
2014	TX777 X LH195	11.33 ±0.93	107%	1.67 ±0.23	83%	1.09	0.27
	TX777 X SS3	10.66 ±0.93	101%	1.73 ±0.23	86%	1.19	0.49
2015	GP286/TX777	10.75 ±0.86	102%	1.83 ±0.17	85%	1.24	0.30

Appendix 6 Continued

Year	Pedigree†	Yield BLUPs‡ Mg ha-1	Percent Check Ave %	Log(Afl) BLUPs	Percent Check Ave %	Type II Stability β	Type III Stability MSE
	(NC300 x Tx714-B/B104-1/CML343)-2-1-B-B-B-B-B-B-B-B-1-B25/TX777	9.98 ±0.86	95%	1.6 ±0.17	74%	1.06	0.10
Appendix 6.	Continued						
	NP2643GT/TX777	9.91 ±0.86	94%	1.89 ±0.17	87%	0.76	0.68
	SGI890/TX777	9.72 ±0.86	92%	1.76 ±0.17	81%	1.36	0.06

†Underlined inbred lines are noted for high yield and/or low aflatoxin. Shaded hybrids are on par with average check yield, and significantly lower in log10(aflatoxin+1) for the given year at p = .05.

‡ BLUP, best linear unbiased predictor.

Appendix 7. BLUPs by year by pedigree

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.			
YEAR	**Pedigree**	**2006 Ck Average**	**9.65**			**2.35**						
2006	DKC69-71	Check	13.4 ±1.26	1*		2.25 ±0.28	14		202.38 ±7.48	14	0 ±7.07	0.75 ±6.43
2006	P31G98	Check	12.6 ±1.03	2*		2.41 ±0.27	34		213.89 ±7.48	1	0 ±7.07	0 ±6.43
2006	(NC300 x Tx714-B/B104-1/CML343)-2-1-B-B-B-B/LH210	Program	12.1 ±1.26	3*	125	2.17 ±0.28	6	92	195.8 ±7.48	24	0.5 ±7.07	1 ±6.43
2006	Croplan 818	Check	11.95 ±1.26	4*		2.25 ±0.28	13		192.18 ±7.48	31	0 ±7.07	0 ±6.43
2006	TV2160Bt	Check	11.73 ±1.26	5*		2.34 ±0.28	25		194.98 ±7.48	26	4 ±7.07	2.25 ±6.43
2006	(CML285/NC300)-B-6-B-B-B-B/LH195	Program	11.7 ±0.96	6*	121	2.18 ±0.27	7*	93	201.55 ±7.48	15	2 ±7.07	7.25 ±6.43
2006	LH195 x LH210	Check	11.5 ±1.26	7*		2.32 ±0.28	18*		205.34 ±7.48	9	0 ±7.07	0.5 ±6.43
2006	DKC67-60	Check	11.25 ±1.26	8*		2.39 ±0.28	33*		210.6 ±7.48	5	4.75 ±7.07	2 ±6.43
2006	S2B73 x NC300	Program	11.17 ±0.96	9*	116	2.07 ±0.27	4*	88	198.27 ±7.48	19	13 ±7.07	8.25 ±6.43
2006	DK697	Check	10.94 ±0.96	10*		2.39 ±0.27	32*		190.04 ±7.48	34	0 ±7.07	2.25 ±6.43
2006	S1W x CML343	Program	10.71 ±0.96	11*	111	2.35 ±0.27	28*	100	209.78 ±7.48	6	3.5 ±7.07	14.25 ±6.43
2006	DW1037 x FR6942HX	Program	10.57 ±0.96	12*	110	2.33 ±0.27	21*	99	199.09 ±7.48	18	0 ±7.07	0.5 ±6.43
2006	(Tx601 x B104-B/FR2128-B x Bd)-2-1-B-B-B-B/LH210	Program	10.51 ±0.96	13*	109	2.13 ±0.27	5*	91	203.69 ±7.48	12	1.25 ±7.07	8 ±6.43
2006	CY-1 x A-2	Program	10.41 ±0.96	14*	108	2.23 ±0.27	11*	95	193.33 ±7.48	29	12.75 ±7.07	18.75 ±6.43
2006	C3CM105-1-B-B-1-1-2 x S2B73	Program	10.21 ±0.96	15	106	2.33 ±0.27	23*	99	204.84 ±7.48	10	12.5 ±7.07	0.5 ±6.43
2006	DW933 x FR6942HX	Program	9.9 ±0.96	16	103	2.54 ±0.27	41	108	208.13 ±7.48	7	7 ±7.07	1 ±6.43
2006	P31G66	Check	9.79 ±0.96	17		2.19 ±0.27	9*		212.24 ±7.48	3	20 ±7.07	23.5 ±6.43
2006	NC300 x S2B73BC	Program	9.63 ±0.96	18	100	2.06 ±0.27	3*	88	201.55 ±7.48	15	8.75 ±7.07	0 ±6.43
2006	LAMA2002-53-5-B/LH195	Program	9.54 ±0.96	19	99	2.25 ±0.27	15*	96	190.37 ±7.48	33	1.75 ±7.07	0 ±6.43
2006	FR1064 x FR6942HX	Program	9.49 ±0.96	20	98	2.46 ±0.27	37	104	199.91 ±7.48	17	1 ±7.07	1.25 ±6.43
2006	DW893 x FR6942HX	Program	9.31 ±0.96	21	97	2.27 ±0.27	16*	97	203.53 ±7.48	13	1.5 ±7.07	1.75 ±6.43
2006	CY-1 x A1-1	Program	9.2 ±0.96	22	95	2.33 ±0.27	22*	99	197.44 ±7.48	21	27.5 ±7.07	40.5 ±6.43
2006	DW1022 x FR6942HX	Program	9.01 ±0.96	23	93	2.49 ±0.27	39	106	198.27 ±7.48	19	4 ±7.07	1 ±6.43
2006	CY-1 x P-2	Program	8.93 ±0.96	24	93	2.31 ±0.27	17*	98	205.66 ±7.48	8	44 ±7.07	15.5 ±6.43
2006	DW1014 x FR6942HX	Program	8.91 ±0.96	25	92	2.41 ±0.27	35*	103	197.44 ±7.48	21	0.5 ±7.07	0 ±6.43
2006	CY-1 x A1R	Program	8.86 ±0.96	26	92	2.32 ±0.29	19*	98	204.84 ±7.48	10	47.5 ±7.07	18.5 ±6.43
2006	(Tx772/CML326)-B-B4-B-B/LH195	Program	8.69 ±0.96	27	90	2.19 ±0.27	10*	93	194.65 ±7.48	28	8.75 ±7.07	2.5 ±6.43
2006	WQ22W x S1W	Program	8.67 ±0.96	28	90	2.32 ±0.27	20*	99	180.18 ±7.48	36	1.75 ±7.07	22.25 ±6.43
2006	SGI912 x FR6942HX	Program	8.54 ±0.96	29	89	2.55 ±0.27	42	109	195.14 ±7.48	25	7.5 ±7.07	2 ±6.43
2006	(Tx811-B x CML 176-B)-B-B-B-B-1-B-B-B/LH195	Program	8.06 ±0.96	31	83	2.18 ±0.27	8*	93	193 ±7.48	30	5.5 ±7.07	3.25 ±6.43
2006	DTP-17B x B110	Program	7.6 ±0.96	32	79	2.24 ±0.27	12*	95	184.12 ±7.48	35	20 ±7.07	1.5 ±6.43
2006	C2A5S4-2-1 x B110	Program	7.4 ±0.96	33	77	2.35 ±0.27	26*	100	190.87 ±7.48	32	14.5 ±7.07	7.25 ±6.43
2006	Mp313E x Mo18W	Program	7.01 ±0.96	34	73	2.04 ±0.27	2*	87	211.26 ±7.48	4	4.75 ±7.07	3.25 ±6.43
2006	DKC 69-70	Check	6.53 ±1.46	35		2.36 ±0.29	31*					
2006	CY-1 x P-27	Program	6.29 ±0.96	36	65	1.95 ±0.29	1*	83	197.28 ±7.48	23	57.25 ±7.07	22.25 ±6.43
2006	GA209 x SC212M	Program	6.29 ±0.96	36	65	2.45 ±0.27	36	104	213.89 ±7.48	1	25.5 ±7.07	14.5 ±6.43

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2006	P31B13	Check	6.13 ±1.46	38		2.36 ±0.29	30*					
2006	BH8913	Check	5.33 ±1.46	39		2.48 ±0.29	38					
2006	P31R88	Check	4.67 ±1.46	40		2.5 ±0.29	40					
2006	(B104-1 x Tx714-B/B110 x FR2128-B)-12-4-B-B-B-B/LH210	Program				2.35 ±0.29	27*	100				
2006	P31R88 Rep III only	Check				2.35 ±0.3	29*					
† Yield for 2006 are *averages* of College Station and Tifton												
*** Top Yielding Group (Fisher's LSD)/Lowest Aflatoxin Group (Fisher's LSD)**												
		2007 Ck Average	**12.44**			**2.45**						
2007	DK697	Check	13.36 ±1.03	1*		2.33 ±0.29	10*		251.88 ±19.08	11	0 ±2.82	0 ±1.59
2007	TV2160Bt	Check	13.32 ±1.28	2*					251.15 ±19.86	14		
2007	P31G66	Check	13.17 ±1.03	3*		2.62 ±0.29	28		264.15 ±19.08	3	0.67 ±2.82	0 ±1.59
2007	P31B13	Check	13.11 ±1.03	4*		2.36 ±0.29	11*		255.36 ±19.08	8	0.67 ±2.82	0 ±1.59
2007	P31G98	Check	12.89 ±1.03	5*		2.52 ±0.29	26		252.68 ±19.08	10	1 ±2.82	0 ±1.59
2007	FR1064 x LH287	Check	11.72 ±1.03	6		2.51 ±0.29	24		237.54 ±19.08	24	0 ±2.82	0 ±1.59
2007	B110 x BR-1	Program	11.55 ±1.03	7	93	2.15 ±0.29	4*	88	256.1 ±19.08	6	3.67 ±2.82	0 ±1.59
2007	(B97x CML 326-B/Tx770 x A645)-1-5-B-B-B/LH195	Program	11.45 ±1.03	8	92	2.26 ±0.29	9*	92	257.97 ±19.08	5	2 ±2.82	0 ±1.59
2007	S2B73 x NC300	Program	11.31 ±1.03	9	91	2.12 ±0.29	3*	86	255.93 ±19.08	7	4 ±2.82	0 ±1.59
2007	FR1064 x TR9352Bt1	Program	11.13 ±1.03	10	89	2.67 ±0.29	30	109	240.3 ±19.08	21	1.33 ±2.82	0 ±1.59
2007	FR1064 x FR4341	Check	11.09 ±1.06	11		2.46 ±0.29	21		247.82 ±19.3	17	0 ±2.82	0 ±1.59
2007	(B110 x FR2128-B/B104-1/CML343)-B-B-6-B-B-B/LH210	Program	10.9 ±1.03	12	88	2.45 ±0.3	20	100	253.73 ±19.08	9	1.67 ±2.82	6.67 ±1.59
2007	FR1064 x LH185	Check	10.87 ±1.03	13		2.38 ±0.29	13		232.18 ±19.08	27	0 ±2.82	0 ±1.59
2007	DW893 x TR9352Bt1	Program	10.75 ±1.03	14	86	2.5 ±0.29	23	102	240.15 ±19.08	22	1.33 ±2.82	0 ±1.59
2007	NC300 x S2B73BC	Program	10.68 ±1.03	16	86	2.03 ±0.29	2*	83	260 ±19.08	4	2 ±2.82	0 ±1.59
2007	DW1022 x TR9352Bt1	Program	10.23 ±1.03	17	82	2.58 ±0.29	27	105	229.49 ±19.08	29	0 ±2.82	0 ±1.59
2007	(CML336-B x Tx772/A645 x Tx770)-7-6-B-B-B/LH195	Program	10.15 ±1.03	18	82	2.18 ±0.29	6*	89	251.69 ±19.08	13	1.33 ±2.82	0.67 ±1.59
2007	GT-P56 x CY-1	Program	10.08 ±1.03	19	81	2.25 ±0.29	8*	92	245.52 ±19.08	18	4.67 ±2.82	0 ±1.59
2007	LH200 x C2A554-4-1-1	Program	10.05 ±1.03	20	81	2.24 ±0.29	7*	91	250.99 ±19.08	15	0 ±2.82	0 ±1.59
2007	Mo18W x Mp313E	Program	9.99 ±1.06	21	80	1.91 ±0.29	1*	78	292.2 ±19.3	1		
2007	DW1014 x TR9352Bt1	Program	9.94 ±1.03	22	80	2.41 ±0.29	16	98	233.5 ±19.08	26	0 ±2.82	0 ±1.59
2007	(CML273 x A632)F7-1b-1-1-B x Tx205	Program	9.77 ±1.03	23	79	2.16 ±0.29	5*	88	244.25 ±19.08	19	11 ±2.82	0 ±1.59
2007	DW997 x TR9352Bt1	Program	9.58 ±1.03	24	77	2.72 ±0.29	31	111	234.16 ±19.08	25	0.67 ±2.82	0 ±1.59
2007	DW1013 x TR9352Bt1	Program	9.45 ±1.03	25	76	2.62 ±0.29	29	107	229.88 ±19.08	28	0 ±2.82	0 ±1.59
2007	WQ22W x S1W	Program	9.16 ±1.03	26	74	2.41 ±0.29	15	98	223.68 ±19.08	30	5.67 ±2.82	2 ±1.59
2007	GT-A2 x CY-1	Program	8.48 ±1.03	27	68	2.45 ±0.29	19	100	251.85 ±19.08	12	11.67 ±2.82	1 ±1.59

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2007	GA209 x SC212M	Program	7.93 ±1.06	28	64	2.52 ±0.29	25	103	276.11 ±19.3	2		
2007	GT-P50 x CY-1	Program	7.57 ±1.03	29	61	2.43 ±0.29	18	99	242.85 ±19.08	20	24 ±2.82	1 ±1.59
2007	GT-A1-1 x CY-1	Program	7.53 ±1.03	30	61	2.46 ±0.29	22	100	239.95 ±19.08	23	13.67 ±2.82	6 ±1.59
2007	(NC300 x Tx714-B/B104-1/CML343)-6-1-B-B/LH210	Program				2.36 ±0.31	12*	96				
2007	S1W x CML343	Program				2.4 ±0.31	14	98				
		2008 Ck Average	**9.26**			**2.60**						
2008	P31P41	Check	10.3 ±0.9	1*		2.59 ±0.27	29		242.38 ±9.06	20	1 ±7.28	0 ±2.55
2008	DK697	Check	9.42 ±0.9	2*		2.49 ±0.28	22		243.16 ±9.42	19	8.5 ±7.28	0 ±2.55
2008	P31B13	Check	9.22 ±1.22	3*		2.68 ±0.31	33		245.86 ±14.38	13		
2008	B110 x BR-1	Program	9.21 ±0.9	4*	99	2.15 ±0.28	5*	83	255.07 ±9.06	6	1.5 ±7.28	0 ±2.55
2008	DW933FL x LH287BT1CCR1	Program	9.15 ±0.9	5*	99	2.39 ±0.27	17	92	244.6 ±9.06	15	2.5 ±7.28	3.5 ±2.55
2008	DW893FL x LH287BT1CCR1	Program	9.09 ±0.9	6*	98	2.47 ±0.27	20	95	237.45 ±9.06	29	2.5 ±7.28	0 ±2.55
2008	DW1022FL x LH287BT1CCR1	Program	9.01 ±0.9	7*	97	2.59 ±0.27	28	99	234.73 ±9.06	33	2 ±7.28	0 ±2.55
2008	DW909FL x LH287BT1CCR1	Program	8.94 ±0.9		97	2.34 ±0.27	13	90	241.94 ±9.06	21	3.5 ±7.28	1 ±2.55
2008	DW997FL x LH287BT1CCR1	Program	8.91 ±0.9		96	2.74 ±0.27	36	105	238.65 ±9.06	28	0 ±7.28	0 ±2.55
2008	P31D58	Check	8.89 ±0.9			2.71 ±0.27	34		245.89 ±9.06	12	0 ±7.28	0 ±2.55
2008	NC300 x S2B73BC	Program	8.84 ±0.9		95	2.28 ±0.27	8	88	254.93 ±9.06	7	0 ±7.28	0 ±2.55
2008	W07-038/LH287	Program	8.57 ±0.9		93	2.59 ±0.27	31	100	239.06 ±9.06	27	0 ±7.28	24 ±2.55
2008	DKC69-71	Check	8.46 ±1.22	14*		2.54 ±0.31	25		245.38 ±14.38	14		
2008	Tx204 x CML32xB104)F7-2-1-b-1-B-2-1-2	Program	8.45 ±0.9	15	91	2.46 ±0.28	19	95	241.47 ±9.06	23	0 ±7.28	0 ±2.55
2008	Y07-118/LH195	Program	8.22 ±0.9	16	89	2.51 ±0.27	23	96	239.86 ±9.06	25	0 ±7.28	0 ±2.55
2008	Y07-111/LH195	Program	8.22 ±0.9	17	89	2.1 ±0.27	4*	81	235.04 ±9.06	32	0 ±7.28	0 ±2.55
2008	Y07-055/LH195	Program	8.18 ±0.9	18	88	2.29 ±0.27	9	88	239.9 ±9.06	24	0 ±7.28	0 ±2.55
2008	Y07-095/LH195	Program	8.1 ±0.9	19	87	2.38 ±0.27	16	92	247.74 ±9.06	11	0 ±7.28	0 ±2.55
2008	CML273xA632)F7-1b-1-1-B x Tx205	Program	7.8 ±0.9	20	84	2.34 ±0.27	14	90	243.38 ±9.06	18	3.5 ±7.28	0 ±2.55
2008	B110xCML343xS1)XB73)F5xMP715-1-4-7-B-1-1-1-1 x C2A554-4-2-1-B-1	Program	7.8 ±0.9	21	84	2.26 ±0.27	7	87	239.24 ±9.06	26	6 ±7.28	1 ±2.55
2008	C3S1B73-1-1-1-1-B-1-1-B x LH287BT1RR2-1	Program	7.78 ±0.9	22	84	2.86 ±0.27	37	110	243.57 ±9.06	17	1 ±7.28	0 ±2.55
2008	FR1064 x FR6942HX1.1	Program	7.63 ±0.9	23	82	2.72 ±0.27	35	105	247.82 ±9.06	10	0 ±7.28	6 ±2.55
2008	AT805 x GT602	Program	7.45 ±0.93	24	81	2.53 ±0.29	24	97	236.71 ±9.82	30	0 ±7.28	0 ±2.55
2008	C3A654-3-2-1-1-1-1-1-1 x LH195Bt1RR2-1	Program	7.45 ±0.9	25	80	2.32 ±0.27	11	89	234.12 ±9.06	34	4 ±7.28	0 ±2.55
2008	AT805 x P50	Program	7.36 ±0.93	26	80	2.6 ±0.29	32	100	244.03 ±9.82	16	10.5 ±7.28	1 ±2.55
2008	B73 x GTP50	Program	7.33 ±0.93	27	79	2.58 ±0.29	27	99	233.27 ±9.82	35	15 ±7.28	0 ±2.55
2008	Mp04:97 x B73	Program	6.97 ±0.9	28	75	2.34 ±0.28	12	90	259.14 ±9.06	5	0 ±7.28	0 ±2.55
2008	Mp04:97 x Mo17	Program	6.66 ±0.9	29	72	2.36 ±0.27	15	91	249.82 ±9.06	9	9 ±7.28	17 ±2.55

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2008	GA209 x SC212M	Program	6.48 ±0.9	30	70	2.42 ±0.27	18	93	249.96 ±9.06	8	1 ±7.28	46 ±2.55
2008	Mo18W x Mp313E	Program	6.4 ±0.9	31	69	2.07 ±0.27	3*	79	278.95 ±9.06	3	0 ±7.28	2.5 ±2.55
2008	GT602 x AT805	Program	6.16 ±0.93	32	67	2.47 ±0.29	21	95	235.99 ±9.82	31	57.5 ±7.28	2 ±2.55
2008	Mp04:97 x Mp07:117	Program	5.89 ±0.9	33	64	2.18 ±0.27	6*	84	267.06 ±9.06	4	1 ±7.28	10 ±2.55
2008	Mp04:97 x Mp313E	Program	5.6 ±0.9	34	61	1.87 ±0.28	2*	72	279.44 ±9.06	2	3 ±7.28	7.5 ±2.55
2008	Mp07:117 x Mp313E	Program	5.39 ±0.91	35	58	1.87 ±0.27	1*	72	283.28 ±9.06	1	1.5 ±7.28	4 ±2.55
2008	P50 x AT805	Program	3.64 ±0.94	36	39	2.54 ±0.29	26	98	230.44 ±9.82	36	0 ±7.28	0 ±2.55
2008	Mo17 x GTP50	Program	2.58 ±0.95	37	28	2.31 ±0.29	10	89	163.51 ±9.82	37	55.5 ±7.28	0 ±2.55
		2009 Ck Average	**9.64**			**2.22**						
2009	P31P41	Check	10.32 ±0.84	1*		2.4 ±0.22	34		274.68 ±11.76	13	0 ±1.13	0 ±2.8
2009	DK697	Check	9.78 ±0.87	2*		1.96 ±0.25	13*		271.86 ±12.05	16	0 ±1.13	0 ±2.8
2009	P31D58	Check	9.75 ±0.84	3*		2.37 ±0.23	30		276.88 ±11.76	12	0 ±1.13	0 ±2.8
2009	S2B73 x NS	Program	9.65 ±0.84	4*	100	2.38 ±0.22	31	107	277.25 ±11.76	10	0 ±1.13	0 ±2.8
2009	CUBA1 x NS	Program	9.49 ±0.84	5*	98	2.13 ±0.22	20	96	287.54 ±11.76	6	0 ±1.13	3 ±2.8
2009	DW1064 x LH287BT1CCR4	Program	9.42 ±0.84	6*	98	2.39 ±0.22	33	108	258.99 ±11.76	27	0 ±1.13	0 ±2.8
2009	C2A632 x NS	Program	9.29 ±0.84	7*	96	2.24 ±0.22	27	101	268.14 ±11.76	19	0 ±1.13	1 ±2.8
2009	P31B13	Check	9.29 ±1.06	8*		2.19 ±0.27	25		272.35 ±14.16	15		
2009	Y07-114/LH287	Program	9.15 ±0.84		95	2.52 ±0.22	38	114	257.3 ±11.76	29	0 ±1.13	0 ±2.8
2009	DW997FL x LH287BT1CCR3	Program	9.06 ±0.84		94	2.43 ±0.22	36	110	258.52 ±11.76	28	0 ±1.13	0 ±2.8
2009	B-H 9014 VT3	Check	9.06 ±1.06	11*		2.16 ±0.27	23		268.81 ±14.16	18		
2009	DW933FL x LH287BT1CCR2	Program	8.83 ±0.84	12	92	2.25 ±0.22	28	102	267.15 ±11.76	20	0 ±1.13	6 ±2.8
2009	(LH195RR2.1xMP313E)BC7P1S1 x LH210	Program	8.82 ±0.84	13	92	2.09 ±0.22	17	94	282.52 ±11.76	9	0 ±1.13	0 ±2.8
2009	DW893FL x LH287BT1CCR1	Program	8.76 ±0.84	14	91	2.39 ±0.22	32	108	253.64 ±11.76	30	0 ±1.13	2.5 ±2.8
2009	DKNaa x SS	Program	8.69 ±0.84	15	90	2.17 ±0.22	24	98	253.21 ±11.76	31	0 ±1.13	0 ±2.8
2009	BMP-1-4-7 x C2A554-4	Program	8.69 ±0.84	16	90	1.88 ±0.22	10*	85	250.25 ±11.76	35	0 ±1.13	0.5 ±2.8
2009	Mp05:115 x LH310	Program	8.63 ±0.84	17	90	1.98 ±0.22	14	89	274.42 ±11.76	14	0 ±1.13	1 ±2.8
2009	B5C2 x NC300	Program	8.58 ±0.84	18	89	1.67 ±0.22	4*	75	286.61 ±11.76	7	0 ±1.13	1.5 ±2.8
2009	GT P50 x B73	Program	8.54 ±0.87	19	89	2.14 ±0.25	21	96	261.02 ±12.05	25	0 ±1.13	0 ±2.8
2009	Mp04:115 x LH195	Program	8.5 ±0.84	20	88	2.11 ±0.22	19	95	261.22 ±11.76	24	0 ±1.13	6 ±2.8
2009	CUBA1 x BR1	Program	8.48 ±0.84	21	88	2.01 ±0.22	16	91	288.09 ±11.76	5	0.5 ±1.13	0 ±2.8
2009	Mp04:97 x LH310	Program	8.25 ±0.84	22	86	1.88 ±0.22	11*	85	292.47 ±11.76	3	0 ±1.13	5.5 ±2.8
2009	Y07-164/LH195	Program	8.2 ±0.84	23	85	1.63 ±0.22	3*	73	248.53 ±11.76	36	0 ±1.13	0 ±2.8
2009	Mp04:107 x LH195	Program	8.07 ±0.84	24	84	1.98 ±0.22	15	89	261.41 ±11.76	23	0 ±1.13	0 ±2.8
2009	Y07-094/LH195	Program	8.07 ±0.84	25	84	2.16 ±0.23	22	97	260.51 ±11.76	26	0 ±1.13	0 ±2.8
2009	AT709xGT601	Program	8.06 ±0.87	26	84	2.43 ±0.25	37	110	271.48 ±12.05	17	0 ±1.13	1 ±2.8

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2009	GT 601 x AT 709	Program	8.06 ±0.87	26	84	2.33 ±0.25	29	105	276.96 ±12.05	11	0 ±1.13	7.5 ±2.8
2009	Y07-104/LH195	Program	8.04 ±0.84	28	83	1.91 ±0.22	12*	86	250.79 ±11.76	34	0 ±1.13	0 ±2.8
2009	GT 601 x DK 888	Program	7.95 ±0.87	29	82	2.1 ±0.25	18	95	297.14 ±12.05	2	3 ±1.13	12 ±2.8
2009	Mp 313E x GT 601	Program	7.94 ±0.87	30	82	1.57 ±0.25	2*	71	288.44 ±12.05	4	3.5 ±1.13	13.5 ±2.8
2009	DK888 x GT 601	Program	7.93 ±0.87	31	82	1.7 ±0.25	6*	77	284.84 ±12.05	8	4.5 ±1.13	18.5 ±2.8
2009	DW893FL x TAMU 2 ((CML288/NC300)-B-9-B1-B-B-B-B))	Program	7.86 ±0.84	32	82	1.82 ±0.22	8*	82	245.85 ±11.76	38	0 ±1.13	0 ±2.8
2009	GT P50 x Mo17	Program	7.82 ±0.87	33	81	2.41 ±0.25	35	109	252.09 ±12.05	32	2 ±1.13	8.5 ±2.8
2009	DW997FL x TAMU 4 ((Tx601 x Tx772)-B-B-20-1-1-B-B-B-B))	Program	7.78 ±0.84	34	81	1.85 ±0.22	9*	83	248.04 ±11.76	37	0 ±1.13	7 ±2.8
2009	Y07-131/Y07-095	Program	7.77 ±0.84	35	81	2.2 ±0.23	26	99	263.99 ±11.76	22	0 ±1.13	0 ±2.8
2009	Mp04:107 x LH310	Program	7.68 ±0.84	36	80	1.8 ±0.22	7*	81	266.27 ±11.76	21	0 ±1.13	0 ±2.8
2009	Mp 04:97 x Mp 04:110	Program	5.94 ±0.85	38	62	1.68 ±0.22	5*	76	251.31 ±11.76	33	1 ±1.13	38 ±2.8
		2010 Ck Average	**8.85**			**2.12**						
2010	DK697	Check	9.56 ±1.2	1*		2.13 ±0.16	37*		260.18 ±10.71	20	0 ±1.47	3.67 ±3.5
2010	DK-7 x SS	Program	9.52 ±1.2	2*	107	2.13 ±0.16	36*	100	277.32 ±10.71	7	0 ±1.47	0.33 ±3.5
2010	P31P41	Check	9.5 ±1.2	3*		2.12 ±0.16	35*		263.14 ±10.71	15	0 ±1.47	0 ±3.5
2010	P31D58	Check	9.35 ±1.2	4*		2.12 ±0.16	33*		261.41 ±10.71	17	0 ±1.47	2 ±3.5
2010	CUBA1 x NS	Program	9.2 ±1.2	5*	104	2.11 ±0.16	29*	100	271.28 ±10.71	10	0.67 ±1.47	0 ±3.5
2010	S2B73BC x NS	Program	8.99 ±1.2	6*	102	2.14 ±0.16	38*	101	266.89 ±10.71	11	0 ±1.47	0 ±3.5
2010	PRA96A x NS	Program	8.79 ±1.2	7*	99	2.12 ±0.16	31*	100	236.27 ±10.71	33	0.67 ±1.47	1 ±3.5
2010	P 31R88	Check	8.79 ±1.43	8*					264.62 ±14.21	14		
2010	Cy-2 x LH132 .FR1064	Program	8.7 ±1.21	9*	98	2.1 ±0.16	20*	99	259.29 ±10.71	21	0 ±1.47	0 ±3.5
2010	CY-2 x SS Tester (LH283 x LH287)	Program	8.64 ±1.4	10*	98							
2010	C2A632-1a x NS	Program	8.62 ±1.2	11*	97	2.1 ±0.16	21*	99	252.89 ±10.71	22	0 ±1.47	1.33 ±3.5
2010	P 31G66	Check	8.57 ±1.43	12*					274.88 ±14.21	8		
2010	HC33 x TR7322	Check	8.46 ±1.43	13*					260.51 ±14.21	19		
2010	P 33M54	Check	8.38 ±1.43	14*					252.3 ±14.21	24		
2010	Mp317 x 50	Program	8.38 ±1.4	15*	95	2.11 ±0.16	24*	99				
2010	WE09-ISO-Pro-111-Yel	Program	8.29 ±1.2		94	2.11 ±0.16	25*	99	237.97 ±10.71	32	0 ±1.47	0.33 ±3.5
2010	Garst 8288	Check	8.22 ±1.43	17*					266.67 ±14.21	12		
2010	Mp317 x 26	Program	8.21 ±1.4	18*	93	2.1 ±0.16	17*	99				
2010	Mp317 x 717	Program	8.19 ±1.4	19*	92	2.09 ±0.16	13*	98				
2010	Mp317 x 55	Program	8.11 ±1.4	20*	92	2.11 ±0.16	26*	99				
2010	Mp317 x 45	Program	7.95 ±1.4	21*	90	2.07 ±0.16	5*	98				
2010	LB08Iso:8039	Program	7.89 ±1.2		89	2.12 ±0.16	32*	100	274.07 ±10.71	9	1 ±1.47	0 ±3.5

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2010	Mp317 x 6	Program	7.64 ±1.4	23	86	2.1 ±0.16	23*	99				
2010	Syn AM 1 (P43) x GP 282	Program	7.6 ±1.2	24	86	2.11 ±0.16	28*	99	246.08 ±10.71	26		0 ±3.5
2010	H08:106x139	Program	7.59 ±1.2	25	86	2.08 ±0.16	8*	98	244.83 ±10.71	28		1.67 ±3.5
2010	Mp317 x 47	Program	7.55 ±1.4	26	85	2.08 ±0.16	10*	98				
2010	GP282 x GT P50	Program	7.52 ±1.2	27	85	2.1 ±0.16	16*	99	245.52 ±10.71	27	0 ±1.47	0.33 ±3.5
2010	Mp317 x 16	Program	7.47 ±1.4	29	84	2.08 ±0.16	9*	98				
2010	CS08-TAC - Yel	Program	7.4 ±1.2	30	84	2.12 ±0.16	34*	100	232.36 ±10.71	34	7 ±1.47	0.67 ±3.5
2010	AT709xGT601	Program	7.38 ±1.2	31	83	2.11 ±0.16	27*	99	261.33 ±10.71	18	1.33 ±1.47	0 ±3.5
2010	Mp313E x Mp317	Program	7.34 ±1.2	32	83	2.05 ±0.16	1*	96	316.7 ±10.71	2	0.67 ±1.47	0.67 ±3.5
2010	WE06-6001-TAC-Yel	Program	7.31 ±1.2	33	83	2.12 ±0.16	30*	100	225.85 ±10.71	35	3 ±1.47	1.67 ±3.5
2010	WE09-ISO-Prp-64-Yel	Program	7.16 ±1.2	34	81	2.1 ±0.16	22*	99	242.88 ±10.71	30	0 ±1.47	0 ±3.5
2010	Syn AM 1 (P43) x GP 280	Program	7.13 ±1.2	35	81	2.14 ±0.16	39*	101	247.95 ±10.71	25	1.33 ±1.47	0.67 ±3.5
2010	Mp313E x Mp715	Program	7.08 ±1.2	36	80	2.05 ±0.16	2*	97	316.97 ±10.71	1	1.67 ±1.47	0.67 ±3.5
2010	CS09-QPMX-059-Wh	Program	7.07 ±1.2	37	80	2.1 ±0.16	19*	99	243.35 ±10.71	29	0.67 ±1.47	0 ±3.5
2010	Mp313E x Mp496	Program	6.98 ±1.2	38	79	2.08 ±0.16	7*	98	300.38 ±10.71	5	2.67 ±1.47	37.33 ±3.5
2010	CS09-QPMX-050-Wh	Program	6.85 ±1.2	39	77	2.08 ±0.16	6*	98	261.99 ±10.71	16	0 ±1.47	46 ±3.5
2010	Mp317 x 494	Program	6.77 ±1.4	40	76	2.06 ±0.16	3*	97				
2010	Mp313E x Mo18W	Program	6.61 ±1.2	41	75	2.09 ±0.16	14*	99	302.65 ±10.71	4	0 ±1.47	0.33 ±3.5
2010	CS09-QPMX-005-Blue	Program	6.54 ±1.2	42	74	2.1 ±0.16	15*	99	238.04 ±10.71	31	0 ±1.47	1.67 ±3.5
2010	CY-5 x SS Testerr (LH283 x LH287)	Program	6.32 ±1.4	43	71							
2010	CY-5 x LH132.FR1064	Program	6.18 ±1.21	44	70	2.14 ±0.16	40*	101	225.41 ±10.71	36	1.67 ±1.47	0 ±3.5
2010	Mp494 x Mp717	Program	5.6 ±1.2	45	63	2.06 ±0.16	4*	97	306.01 ±10.71	3	0.67 ±1.47	6.33 ±3.5
2010	Mp715 x Mp717	Program	5.02 ±1.2	46	57	2.1 ±0.16	18*	99	297.91 ±10.71	6	0 ±1.47	18 ±3.5
2010	NC300 x Mp715	Program	4.31 ±1.2	47	49	2.09 ±0.16	12*	98	265.64 ±10.71	13	0.67 ±1.47	20.67 ±3.5
		2011 Ck Average	**7.67**			**2.40**						
2011	S2B73BC x NS	Program	8.5 ±1.22	1*	111	2.56 ±0.18	33	107	232.02 ±9.08	20	0.33 ±1.03	0 ±0
2011	P31P41	Check	8.29 ±1.22	2*		2.4 ±0.18	12		238.72 ±9.08	14	0 ±1.03	0 ±0
2011	P31G98	Check	8.17 ±1.22	3*		2.27 ±0.18	6*		248.19 ±9.08	3	0 ±1.03	0 ±0
2011	DK697	Check	8.08 ±1.22	4*		2.41 ±0.18	17		244.13 ±9.08	9	0 ±1.03	0 ±0
2011	P31G96	Check	8.04 ±1.35	5*		2.41 ±0.21	20*		242.78 ±11.14	13		
2011	PR96A x NS	Program	8 ±1.22	6*	104	2.78 ±0.18	37	116	245.35 ±9.08	5	0 ±1.03	0 ±0
2011	DKC67-87	Check	7.99 ±1.35	7*		2.52 ±0.21	32		233.72 ±11.14	19		
2011	C2A632 x NS	Program	7.83 ±1.22	8*	102	2.45 ±0.18	24	102	244.98 ±9.08	7	0 ±1.03	0 ±0
2011	BH8928VTTP	Check	7.82 ±1.35	9*		2.18 ±0.21	4*		230.42 ±11.14	23		
2011	P31G66	Check	7.72 ±1.31	11*					250.04 ±10.47	2		
2011	CUBA1 x NS3	Program	7.61 ±1.22	12*	99	2.2 ±0.18	5*	92	245.06 ±9.08	6	0.33 ±1.03	0 ±0

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2011	P33D49	Check	7.36 ±1.35	13*		2.43 ±0.21	21*		226.31 ±11.14	25		
2011	CUBA1 x NS	Program	7.28 ±1.22	14	95	2.65 ±0.18	36	110	231.35 ±9.08	21	0 ±1.03	0 ±0
2011	BR-1 x SS	Program	7.12 ±1.22	15	93	2.13 ±0.18	1*	89	242.96 ±9.08	12	0 ±1.03	0 ±0
2011	BMP-14-7 x A2A554-4	Program	7.06 ±1.22	16	92	2.43 ±0.18	22	101	255.42 ±9.08	1	1.33 ±1.03	0 ±0
2011	CUBA1 x NS2	Program	7.04 ±1.22	17	92	2.45 ±0.18	25	102	237.79 ±9.08	15	0 ±1.03	0 ±0
2011	((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B2/SS	Program	6.87 ±1.22	18	90	2.32 ±0.18	7*	97	246.78 ±9.08	4	0 ±1.03	0 ±0
2011	ArgnetineFlintyComposite-C(1)-14-B-B/SS	Program	6.85 ±1.22	19	89	2.41 ±0.18	18	100	233.75 ±9.08	18	0 ±1.03	0 ±0
2011	Mp494 x 50	Check	6.83 ±1.35	20		2.5 ±0.21	30		244.42 ±11.14	8		
2011	CY1 x NC262B	Program	6.81 ±1.22	21	89	2.5 ±0.18	29	104	226.11 ±9.08	26	0 ±1.03	0 ±0
2011	Mp 04:94 x PHW79	Program	6.72 ±1.35	22	88	2.44 ±0.21	23*	101				
2011	ArgnetineFlintyComposite-C(1)-15-B1-B/SS	Program	6.58 ±1.22	23	86	2.49 ±0.18	28	104	225.77 ±9.08	27	2 ±1.03	0 ±0
2011	Mp317 x K0679y	Check	6.44 ±1.35	24		2.52 ±0.21	31		237.01 ±11.14	17		
2011	Mp 04:115 x PHW 79	Program	6.39 ±1.35	25	83	2.63 ±0.21	35	109				
2011	Mp 04:87 x PHW79	Program	6.37 ±1.35	26	83	2.48 ±0.21	27*	103				
2011	((LAMA2002-12-1-B/(CML 325/B104)-B-1-B-B-B-B)-B-B2-3-2-B-B)xLH132	Program	6.28 ±1.22	27	82	2.47 ±0.18	26	103	224.1 ±9.08	29	0.67 ±1.03	0 ±0
2011	GP282 x GT603	Program	6.26 ±1.22	28	82	2.39 ±0.18	11*	100	231.34 ±9.08	22	0 ±1.03	0 ±0
2011	Mp 04:110 x PHW 79	Program	6.26 ±1.35	29	82	2.41 ±0.21	15*	100				
2011	AT709xGT601	Program	6.15 ±1.22	30	80	2.41 ±0.18	16	100	243.61 ±9.08	10	0.67 ±1.03	0 ±0
2011	Mp 719 x PHW79	Program	6.05 ±1.35	31	79	2.18 ±0.21	3*	91				
2011	((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1/NSS	Program	6.02 ±1.22	32	78	2.58 ±0.18	34	107	219.26 ±9.08	31	0 ±1.03	0 ±0
2011	Mp 718 x PHW79	Program	6 ±1.35	33	78	2.16 ±0.21	2*	90				
2011	((LAMA2002-2-5-B/(CML285/B104)-B-4-B-B-B-B)-B-B2-2-3-B-B)xLH132	Program	5.88 ±1.22	34	77	2.4 ±0.18	13*	100	226.36 ±9.08	24	2.67 ±1.03	0 ±0
2011	((CML288/NC300)-B-9-B1-B-B-B-B-B-B-B)xLH132	Program	5.68 ±1.22	35	74	2.34 ±0.19	9*	97	220.56 ±9.08	30	2.67 ±1.03	0 ±0
2011	Lo964 x GT603	Program	5.54 ±1.22	36	72	2.39 ±0.18	10*	99	224.2 ±9.08	28	5.67 ±1.03	0 ±0
2011	GT603 x DK888N11Fls3,2141-2-34-B-2-1	Program	5.48 ±1.22	37	71	2.33 ±0.18	8*	97	237.12 ±9.08	16	1.67 ±1.03	0 ±0
2011	Tx-WX11-9	Program				2.4 ±0.22	14*	100				
		2012 Ck Average	**9.74**			**2.08**						
2012	P31P41	Check	10.56 ±1.2	1*		2.32 ±0.23	37		271.92 ±7.14	10	1 ±7.17	0 ±3.81
2012	P31G98	Check	9.93 ±1.2	2*		2.04 ±0.23	23		276.91 ±7.14	7	8 ±7.17	9.33 ±3.81
2012	BH8910RR/HX	Check	9.74 ±1.2	3*		2.12 ±0.23	29		285.25 ±7.14	2	41.33 ±7.17	2.33 ±3.81
2012	DK697	Check	9.64 ±1.2	4*		1.96 ±0.23	17		270.31 ±7.14	11	7 ±7.17	4 ±3.81
2012	BH8740VTTP	Check	9.59 ±1.2	5*		2.06 ±0.23	28		268.37 ±7.14	13	1.33 ±7.17	0 ±3.81
2012	P31D58	Check	9.49 ±1.3	6*					260.55 ±8.84	23		
2012	BH9051RR	Check	9.24 ±1.2	7		1.97 ±0.23	18		253.24 ±7.14	26	8 ±7.17	2.67 ±3.81

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2012	Tx-WX12-01	Program	9.23 ±1.2	8	95	2.22 ±0.23	33	107	277.14 ±7.14	6	8.33 ±7.17	11 ±3.81
2012	Tx-WX12-02	Program	9.04 ±1.2	9	93	2.69 ±0.23	43	130	266.94 ±7.14	16	12.33 ±7.17	7.67 ±3.81
2012	Tx-WX12-07	Program	8.95 ±1.2	10	92	1.88 ±0.23	13	91	274.52 ±7.14	8	1.33 ±7.17	14.33 ±3.81
2012	TZAR106 X LH51	Program	8.9 ±1.36	11*	91	1.33 ±0.31	1*	64				
2012	TZAR106 X LH132	Program	8.8 ±1.36	12	90	1.45 ±0.31	5*	70				
2012	Tx-WX12-03	Program	8.55 ±1.2	13	88	2.47 ±0.23	41	119	259.83 ±7.14	24	1.67 ±7.17	0.67 ±3.81
2012	TZAR101 X LH132	Program	8.48 ±1.36	14	87	2.05 ±0.31	25	99				
2012	((Tx741) ; LAMA2002-42-B-B-B-B-B3) X SS3	Program	8.46 ±1.2	15	87	1.47 ±0.23	6*	71	250.84 ±7.14	27	1.67 ±7.17	0 ±3.81
2012	Tx-WX12-04	Program	8.43 ±1.2	16	87	2.4 ±0.23	40	115	267.89 ±7.14	14	6.33 ±7.17	4.33 ±3.81
2012	TZAR102 X LH51	Program	8.41 ±1.36	17	86	2.01 ±0.31	20	97				
2012	LH132 x SynAMP43	Program	8.36 ±1.2	18	86	2.05 ±0.23	24	98	260.79 ±7.14	22	6.67 ±7.17	2.67 ±3.81
2012	TZAR103 X LH51	Program	8.33 ±1.36	19	85	1.36 ±0.31	3*	65				
2012	(B97x CML 326-B/Tx770 x A645)-2-2-B-B-B-B-B-B-B-B X SS2	Program	8.3 ±1.2	20	85	2.03 ±0.23	22	98	264.33 ±7.14	19	25.67 ±7.17	2 ±3.81
2012	Tx-WX12-05	Program	8.28 ±1.2	21	85	2.37 ±0.23	39	114	264.04 ±7.14	20	13 ±7.17	3.67 ±3.81
2012	[(Mp494 X GEMN-013) X (Mp717 X GEMS-0074)]	Program	8.12 ±1.28	22	83	1.34 ±0.25	2*	64	269.16 ±9.61	12	26.33 ±7.17	3.67 ±3.81
2012	Mp494 X GEMN-0130	Program	8.07 ±1.28	23	83	1.55 ±0.26	7*	75	284.46 ±9.61	3	4.67 ±7.17	6.33 ±3.81
2012	Tx-WX12-06	Program	8.03 ±1.2	24	82	2.06 ±0.23	27	99	247.92 ±7.14	28	4.67 ±7.17	1 ±3.81
2012	Mp313E x GEMN-0157	Program	7.98 ±1.3	25	82				280.14 ±8.84	5		
2012	(BS13(S)C8-34-1-B-B-B-B-B-B-B-B-B) X NSS2	Program	7.89 ±1.2	26	81	1.97 ±0.23	19	95	241.81 ±7.14	33	3.67 ±7.17	1 ±3.81
2012	TZAR104 X LH51	Program	7.79 ±1.36	27	80	2.34 ±0.31	38	112				
2012	TZAR104 X LH132	Program	7.77 ±1.36	28	80	2.26 ±0.31	35	109				
2012	BS13(S)C8-11-1-B-B-B-B-B-B-B-B-B X NSS2	Program	7.7 ±1.2	29	79	2.01 ±0.23	21	97	241.82 ±7.14	32	11 ±7.17	1.67 ±3.81
2012	[(Mp494 X NEI9008:S17c21-091-001-B-B) X (Mp313E X GEMN-0157)]	Program	7.58 ±1.22	31	78	1.72 ±0.26	10	83	299.38 ±7.69	1	46 ±7.17	9.67 ±3.81
2012	TZAR101 X LH51	Program	7.45 ±1.36	32	77	2.25 ±0.31	34	108				
2012	LH132 x GTA2R	Program	7.45 ±1.2	33	76	2.15 ±0.23	31	103	266.95 ±7.14	15	8.67 ±7.17	4.67 ±3.81
2012	CY1 x GT603	Program	7.34 ±1.2	34	75	1.93 ±0.23	16	93	241.12 ±7.14	34	32 ±7.17	13.33 ±3.81
2012	LH51 x SynAMP43	Program	7.34 ±1.2	35	75	2.5 ±0.23	42	120	266.21 ±7.14	18	6.33 ±7.17	2.67 ±3.81
2012	LAMA2002-46-3-B-B-B-B-B X LH82	Program	7.31 ±1.22	36	75	2.19 ±0.25	32	105	242.1 ±7.69	31	7.67 ±7.17	1.67 ±3.81
2012	Hi27 x GT603	Program	7.21 ±1.2	37	74	1.7 ±0.23	9*	82	261.53 ±7.14	21	9.33 ±7.17	6.33 ±3.81
2012	Hi33 x Ni7077-6	Program	7.19 ±1.2	38	74	1.89 ±0.23	14	91	274.29 ±7.14	9	34.33 ±7.17	0.67 ±3.81
2012	Mp313E X GEMS-0074	Program	7 ±1.28	39	72	1.58 ±0.26	8*	76	266.38 ±9.61	17	38.67 ±7.17	3.33 ±3.81
2012	HBA x GT603	Program	6.94 ±1.2	40	71	1.93 ±0.23	15	93	258.23 ±7.14	25	44.67 ±7.17	6 ±3.81
2012	LAMA2002-46-3-B-B-B-B-B/LH82	Program	6.65 ±1.36	41	68	1.76 ±0.31	11*	85	243.92 ±9.61	29		
2012	SS2 X ((B104-1 x Tx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1-1-B9	Program	6.53 ±1.2	42	67	2.28 ±0.23	36	110	243.28 ±7.14	30	6.67 ±7.17	1.33 ±3.81
2012	((B104-1 x Tx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1-1-B9 X SS	Program	5.09 ±1.2	43	52	2.13 ±0.23	30	103	196.84 ±7.14	35	4.33 ±7.17	0 ±3.81
2012	DKC 66-23	Check				2.06 ±0.35	26					

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2012	DKC 67-88	Check				1.88 ±0.35	12*					
		2013 Ck Average	**9.34**			**2.07**						
2013	Tx777 X SS2	Program	10 ±0.75	1*	107	1.79 ±0.17	11	86	260.02 ±7.48	7	5 ±4.16	0 ±8.39
2013	P31G98	Check	9.97 ±0.76	2*		2.15 ±0.17	26		263.61 ±7.48	5	2.5 ±4.16	0 ±8.39
2013	Tx777 X SS3	Program	9.95 ±0.75	3*	106	1.64 ±0.17	8	79	280.52 ±7.48	1	0 ±4.16	8 ±8.39
2013	P31P41	Check	9.78 ±0.75	4*		2.34 ±0.17	38		246.03 ±7.48	28	0 ±4.16	0 ±8.39
2013	BH8910RR/HX	Check	9.44 ±0.75	5*		1.95 ±0.17	20		273.4 ±7.48	2	10.5 ±4.16	7 ±8.39
2013	SS1 X (CML450-B/Tx110)-B-3-B-1-B-B-1-1-B18	Program	9.32 ±0.75	6*	100	1.82 ±0.17	13	88	255.5 ±7.48	12	0 ±4.16	0 ±8.39
2013	DK697	Check	9.28 ±0.75	7*		2.03 ±0.17	23		253.58 ±7.48	17	0 ±4.16	0 ±8.39
2013	SS2 X (LAMA2002-22-1-B-B-B-B/LAMA2002-1-5-B-B-B-B)-2-1-B-1-1-1-B19	Program	9.18 ±0.75	8*	98	2.02 ±0.17	22	98	250.83 ±7.48	21	4.5 ±4.16	2.5 ±8.39
2013	(LAMA2002-35-2-B-B-B-B/CG44)-1-3-B-1-1-B24 X SS3	Program	9.11 ±0.75	9*	98	1.91 ±0.17	17	92	253.04 ±7.48	18	0 ±4.16	0 ±8.39
2013	BH8740VTTP	Check	9.02 ±0.75	10*		1.93 ±0.17	18		250.96 ±7.48	20	1.5 ±4.16	0 ±8.39
2013	SS1 X (LAMA2002-61-2-BB/LAMA2002-53-5-BB)-B*5-1-B6-1-B16	Program	9.02 ±0.75	11*	97	1.61 ±0.17	7	78	253.91 ±7.48	16	3.5 ±4.16	4 ±8.39
2013	LAMA2002-58-3-B-B-B-B-B-B-1-B19 X NSS2	Program	8.98 ±0.75	12*	96	1.75 ±0.17	10	84	241.53 ±7.48	33	2 ±4.16	0 ±8.39
2013	CUBA1 x NS	Program	8.74 ±0.75	14	94	1.81 ±0.17	12	87	260.72 ±7.48	6	1.5 ±4.16	14 ±8.39
2013	CUBA1TEO43 x NS	Program	8.72 ±0.75	15	93	2.28 ±0.17	35	110	246.27 ±7.48	27	6.5 ±4.16	4 ±8.39
2013	BH9051RR	Check	8.56 ±0.75	16		2.04 ±0.17	24		246.27 ±7.48	26	2.5 ±4.16	0 ±8.39
2013	CUBA1TEO51-1 x NS	Program	8.49 ±0.75	17	91	2.27 ±0.17	33	109	256.71 ±7.48	11	3.5 ±4.16	10.5 ±8.39
2013	CUBA1TEO42 x NS	Program	8.47 ±0.75	18	91	2.23 ±0.17	30	108	244.34 ±7.48	30	1.5 ±4.16	20 ±8.39
2013	CUBA1TEO62 xNS	Program	8.41 ±0.75	19	90	2.19 ±0.17	29	106	247.11 ±7.48	24	4.5 ±4.16	15 ±8.39
2013	(LAMA2002-35-2-B-B-B-B/CG44)-1-3-B-B14 X SS2	Program	8.39 ±0.75	20	90	1.84 ±0.17	15	89	249.79 ±7.48	22	10 ±4.16	14 ±8.39
2013	CUBA1TEO30 x NS	Program	8.39 ±0.76	21	90	2.16 ±0.17	27	104	254.81 ±7.48	15	6 ±4.16	45.5 ±8.39
2013	CUBA1TEO41 x NS	Program	8.36 ±0.76	22	89	2.31 ±0.17	37	111	245.33 ±7.48	29	3 ±4.16	6 ±8.39
2013	GP280 x GT603	Program	8.32 ±0.76	23	89	2.24 ±0.17	32	108	258.62 ±7.48	8	0 ±4.16	6.5 ±8.39
2013	B3C2B5-19/20 x NS	Program	8.24 ±0.76	24	88	2.28 ±0.17	34	110	247.35 ±7.48	23	9.5 ±4.16	14 ±8.39
2013	CUBA1TEO67 x NS	Program	8.2 ±0.75	25	88	2.47 ±0.17	39	119	255.02 ±7.48	13	0 ±4.16	6 ±8.39
2013	GT-A2R x B73	Program	8.13 ±0.75	26	87	2.01 ±0.17	21	97	264.39 ±7.48	4	14.5 ±4.16	8 ±8.39
2013	(CML288/NC300)-B-9-B1-B-B-B-B-B-B-B-B14 X LH195 (GRIN-PI)	Program	7.99 ±0.75	27	85	1.45 ±0.17	5*	70	241.58 ±7.48	32	0 ±4.16	0 ±8.39
2013	Mp 313E x NC 388	Program	7.95 ±1	28	85	1.44 ±0.25	4*	69				
2013	GP282 X GT603	Program	7.84 ±0.75	29	84	1.72 ±0.17	9	83	252.56 ±7.48	19	11 ±4.16	6.5 ±8.39

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2013	SS4 X (((B104/NC300)x(CML 415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B	Program	7.84 ±0.75	30	84	2.23 ±0.17	31	108	238.87 ±7.48	35	3.5 ±4.16	0 ±8.39
2013	GEMS 0005-2-1B X Hi27bs	Program	7.59 ±0.75	31	81	1.41 ±0.17	3*	68	257.39 ±7.48	10	2 ±4.16	0 ±8.39
2013	Mp 313E x Mp 717	Program	7.53 ±1	32	81	1.35 ±0.25	2*	65				
2013	Hi63xNC466	Program	7.41 ±0.75	33	79	1.88 ±0.17	16	91	254.89 ±7.48	14	1.5 ±4.16	27.5 ±8.39
2013	HBA1-1-1-1B X GT-603	Program	7.32 ±0.76	34	78	2.19 ±0.17	28	105	258.04 ±7.48	9	18.5 ±4.16	33 ±8.39
2013	GTA2R-1B-1B X TUN 85	Program	7.13 ±0.75	35	76	1.84 ±0.17	14	89	243.99 ±7.48	31	72 ±4.16	3 ±8.39
2013	Mp 313E x Mp 719	Program	6.94 ±1	36	74	1.02 ±0.25	1*	49				
2013	GT-A2R x Mo17	Program	6.74 ±0.75	37	72	1.95 ±0.17	19	94	241.48 ±7.48	34	61 ±4.16	12 ±8.39
2013	GTA2R-1B-1B X SC212M	Program	6.49 ±0.75	38	69	2.3 ±0.17	36	111	268.73 ±7.48	3	32.5 ±4.16	41.5 ±8.39
2013	TUN18-2 x GT603	Program	6.18 ±0.75	39	66	2.14 ±0.17	25	103	246.95 ±7.48	25	28 ±4.16	41.5 ±8.39
2013	CUBA1TEO21 x NS	Program	4.38 ±0.76	40	47	2.85 ±0.17	40	137	216.32 ±7.48	36	13.5 ±4.16	14 ±8.39
		2014 Ck Average	**10.55**			**2.02**						
2014	P1745R	Check	11.38 ±0.93	2*		2.21 ±0.23	39		234.47 ±9.11	15	4 ±2.94	0.33 ±1.38
2014	Tx777\X\LH195	Program	11.33 ±0.93	3*	107	1.67 ±0.23	6*	83	229.98 ±9.11	18	0 ±2.94	0 ±1.38
2014	BH8910RR/HX	Check	11.21 ±0.93	4*		2.05 ±0.23	35		250.84 ±9.11	6	0 ±2.94	0 ±1.38
2014	P2088R	Check	11.17 ±0.93	5*		2.39 ±0.23	41		240.25 ±9.11	10	3 ±2.94	0 ±1.38
2014	P31P41	Check	10.83 ±0.93	6*		2.17 ±0.23	38		229.65 ±9.11	22	1 ±2.94	0 ±1.38
2014	DK697	Check	10.82 ±0.93	7*		2.04 ±0.23	33		229.58 ±9.11	23	0 ±2.94	0 ±1.38
2014	SS2\X\(CML450-B/Tx110)-B-3-B-1-B-B-1-1-B18	Program	10.8 ±0.93	8*	102	1.94 ±0.23	23	96	243.61 ±9.11	8	0.33 ±2.94	0 ±1.38
2014	Tx777 X SS3	Program	10.66 ±0.93	9	101	1.73 ±0.23	9	86	227.37 ±9.11	27	0 ±2.94	0 ±1.38
2014	P31G98	Check	10.58 ±0.93	10		2 ±0.23	29		233.54 ±9.11	17	0 ±2.94	0 ±1.38
2014	LAMA2002-58-3-B-B-B-B-B-B-1-B19\X\NSS1	Program	10.5 ±0.93	11	100	1.88 ±0.23	19	93	225.72 ±9.11	29	0 ±2.94	0 ±1.38
2014	SS1\X\(LAMA2002-10-1-B/(CML288/NC300)-B-9-B1-B-B-B)-B-B-1-3-B-1-B	Program	10.37 ±0.93	12	98	2.02 ±0.23	31	100	235.65 ±9.11	14	0 ±2.94	0 ±1.38
2014	BR-1 x SS1	Program	10.3 ±0.93	13	98	1.99 ±0.23	28	99	244.85 ±9.11	7	0 ±2.94	0 ±1.38
2014	(LAMA2002-22-1-B-B-B-B/LAMA2002-1-5-B-B-B-B)-2-1-B-1-1-1-B19-B18\X\LH195	Program	9.94 ±0.93	14	94	1.83 ±0.23	16	91	220.63 ±9.11	36	0 ±2.94	0 ±1.38
2014	BH9051RR	Check	9.91 ±0.93	15		1.8 ±0.23	15		218.41 ±9.11	37	2 ±2.94	0 ±1.38
2014	SS1 x C2A5-1	Program	9.85 ±0.93	16	93	1.97 ±0.23	27	98	225.69 ±9.11	30	1 ±2.94	0 ±1.38
2014	SS1\X\(LAMA2002-35-2-B-B-B-B/CG44)-1-3-B-B14-B10	Program	9.83 ±0.93	17	93	1.79 ±0.23	12	89	217.43 ±9.11	38	4.67 ±2.94	1 ±1.38
2014	BH8740VTTP	Check	9.81 ±0.93	18		1.79 ±0.23	13		229.88 ±9.11	19	9.33 ±2.94	0 ±1.38
2014	SS1 x Tx208	Program	9.64 ±0.93	19	91	2.22 ±0.23	40	110	225.19 ±9.11	31	0.33 ±2.94	0 ±1.38
2014	(LAMA2002-23-1-B-B/LAMA2002-11-1-B-B)-B-B-B-B-B-1-B6\X\SS1	Program	9.54 ±0.93	20	90	2.04 ±0.23	32	101	216.76 ±9.11	39	0 ±2.94	0 ±1.38
2014	SS1 x C2A5-4	Program	9.45 ±0.93	21	90	1.87 ±0.23	18	93	229.17 ±9.11	24	10 ±2.94	1 ±1.38
2014	DK68-04	Check	9.22 ±0.93	22		1.69 ±0.23	7		207.91 ±9.11	40	9.33 ±2.94	1 ±1.38
2014	SS1 x C2A5-2	Program	9.2 ±0.93	23	87	1.96 ±0.23	25	97	229.72 ±9.11	21	7.33 ±2.94	0 ±1.38

Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.		Ht. Rank	Stm Lodge	Rt Lodge
2014	FAW 1430 x NC358	Program	9.15 ±0.93	24	87	1.78 ±0.23	11	88	229.81 ±9.11	20	0 ±2.94	1 ±1.38	
2014	PHG39 x DK888	Program	9.05 ±0.93	25	86	2.01 ±0.23	30	100	239.93 ±9.11	12	1 ±2.94	0.33 ±1.38	
2014	GRACE E-5 (E-1) x DK888	Program	8.96 ±0.93	26	85	1.86 ±0.23	17	92	227.12 ±9.11	28	2 ±2.94	1 ±1.38	
2014	SYN AM P43 x DK888	Program	8.87 ±0.93	27	84	1.91 ±0.23	21	95	222.82 ±9.11	33	0.33 ±2.94	0 ±1.38	
2014	Oh43 x FAW 1430	Program	8.81 ±0.93	28	84	2.07 ±0.23	36	103	239.94 ±9.11	11	0.33 ±2.94	0 ±1.38	
2014	Mp13:9031 x Mp13:9032	Program	8.69 ±0.93	29	82	1.45 ±0.23	3*	72	254.01 ±9.11	4	0 ±2.94	0 ±1.38	
2014	Hi63xNC466	Program	8.69 ±0.93	30	82	1.91 ±0.23	20	95	228.54 ±9.11	25	0 ±2.94	0 ±1.38	
2014	GT A2 R 1B 1B x DK888	Program	8.6 ±0.93	31	82	1.97 ±0.23	26	98	235.67 ±9.11	13	1.67 ±2.94	3 ±1.38	
2014	SS1 x C2A5-3	Program	8.54 ±0.93	33	81	1.93 ±0.23	22	96	228.31 ±9.11	26	14 ±2.94	1 ±1.38	
2014	GEMS-0028-2-1 x GT603	Program	8.53 ±0.93	34	81	1.45 ±0.23	2*	72	234.24 ±9.11	16	1 ±2.94	0 ±1.38	
2014	Mp13:9011 x Mp13:9012	Program	8.33 ±0.93	35	79	1.6 ±0.23	4*	80	265.46 ±9.11	2	0.67 ±2.94	7.67 ±1.38	
2014	B5C2RM-45-1-1 x NS1	Program	8.3 ±0.93	36	79	2.07 ±0.23	37	103	221.76 ±9.11	35	3 ±2.94	1 ±1.38	
2014	Mp13:9035 x Mp13:9036	Program	7.8 ±0.93	37	74	1.71 ±0.23	8	85	264.11 ±9.11	3	0.67 ±2.94	0.67 ±1.38	
2014	Mp13:9025 x Mp13:9026	Program	7.77 ±0.93	38	74	1.31 ±0.23	1*	65	267.84 ±9.11	1	0.67 ±2.94	0.67 ±1.38	
2014	Hi31 x GT603	Program	7.44 ±0.93	39	71	1.62 ±0.23	5*	80	222.15 ±9.11	34	24.33 ±2.94	0.33 ±1.38	
2014	((Tx740/Mp715)//(Tx772/Mp313))-#/((Tx772/Mp715)//(Tx740/Mp313E))-#	Program	6.49 ±0.93	40	62	1.76 ±0.23	10	87	253.1 ±9.11	5	1 ±2.94	0 ±1.38	
2014	NC358 X NC350	Check				1.79 ±0.27	14						
		2015 Ck Average	**10.53**			**2.17**							
2015	Terral 28R20	Check	12.23 ±0.86	1*		2.14 ±0.17	32		284.06 ±12.34	10	1.33 ±2.86	2 ±9.61	
2015	P2088R	Check	11.48 ±0.86	2*		2.56 ±0.17	40		272.96 ±12.34	15	2 ±2.86	5.33 ±9.61	
2015	P1745R	Check	11.46 ±0.86	3*		2.38 ±0.17	38		270.46 ±12.34	17	1.33 ±2.86	4.33 ±9.61	
2015	GP474GT/Tx777	Program	11.33 ±0.88	4*	108	2.06 ±0.17	26	95	268.19 ±12.34	19	0 ±2.86	0 ±9.61	
2015	GP286/Tx777	Program	10.75 ±0.86	5	102	1.83 ±0.17	13*	85	263.96 ±12.34	27	0 ±2.86	6.67 ±9.61	
2015	P31G98	Check	10.72 ±0.86	6		2.11 ±0.17	30		276.99 ±12.34	12	1.33 ±2.86	5.33 ±9.61	
2015	P31P41	Check	10.64 ±0.86	7		2.3 ±0.17	37		262.63 ±12.34	29	0.67 ±2.86	0 ±9.61	
2015	DK697	Check	10.59 ±0.86	8		1.94 ±0.17	20*		270.72 ±12.34	16	0 ±2.86	1.33 ±9.61	
2015	DK64-69	Check	10.29 ±0.86	9		2.25 ±0.17	36		245.12 ±12.34	38	0 ±2.86	0 ±9.61	
2015	NC_CK1	Check	10.08 ±1.04	10					265.37 ±13.47	23			
2015	(NC300 x Tx714-B/B104-1/CML343)-2-1-B-B-B-B-B-B-B-1-B25/Tx777	Program	9.98 ±0.86	11	95	1.6 ±0.17	3*	74	260.6 ±12.34	33	1.33 ±2.86	8 ±9.61	
2015	NP2643GT/Tx777	Program	9.91 ±0.86	12	94	1.89 ±0.17	16*	87	263.92 ±12.34	28	0.67 ±2.86	14.67 ±9.61	
2015	ANTIGO4 x SS1	Program	9.87 ±0.86	13	94	2.1 ±0.17	29	97	262.23 ±12.34	32	3.33 ±2.86	9 ±9.61	
2015	CUBA1 x NS1	Program	9.75 ±0.86	14	93	2.07 ±0.17	27	96	267.77 ±12.34	20	0.67 ±2.86	24.33 ±9.61	
2015	SGI890/Tx777	Program	9.72 ±0.86	15	92	1.76 ±0.17	10*	81	274.66 ±12.34	13	1.67 ±2.86	3 ±9.61	
2015	GP280GT/Tx777	Program	9.68 ±0.86	16	92	1.91 ±0.17	18*	88	274.06 ±12.34	14	1.33 ±2.86	3 ±9.61	
2015	LH210 X GT1309	Program	9.43 ±0.88	17	90	1.67 ±0.17	6*	77	286.77 ±12.34	8	24.33 ±2.86	23 ±9.61	

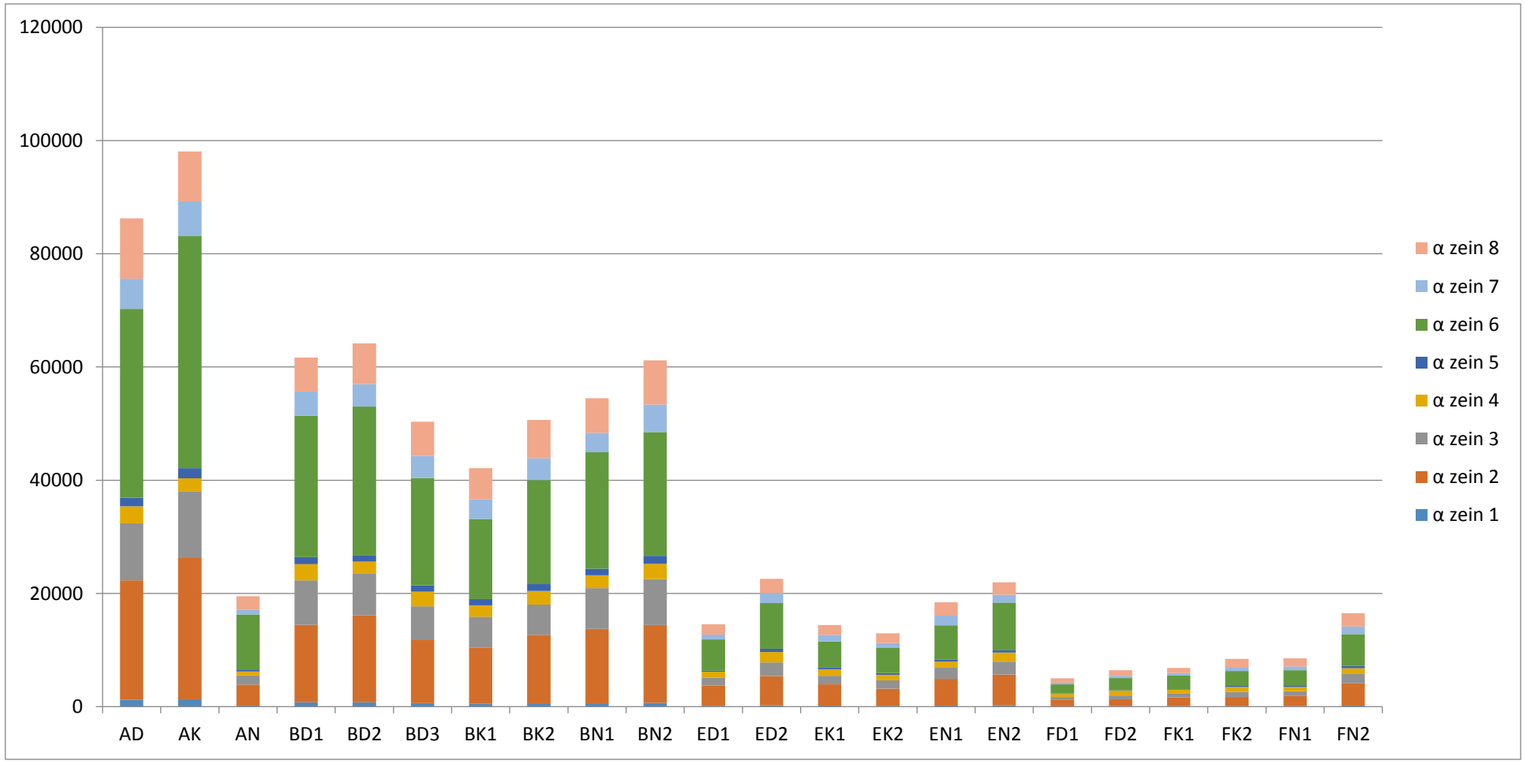
Appendix 7 continued.

YEAR	Pedigree	Program or Check	Yield BLUPs† t ha^{-1}	Yield rank	Percent of check avg	Log(Afl+1) BLUPs	Log(Afl) rank	Percent of check avg	Plt ht BLUPs cm.	Ht. Rank	Stm Lodge	Rt Lodge
2015	ANTIGO6 x SS1	Program	9.34 ±0.86	18	89	2.16 ±0.17	33	100	267.75 ±12.34	21	4.67 ±2.86	2.33 ±9.61
2015	ANTIGO19/20 x SS1	Program	9.19 ±0.86	20	87	2.21 ±0.17	35	102	264.54 ±12.34	25	12 ±2.86	31.67 ±9.61
2015	Zm 521 E-1 X B73	Program	9.06 ±0.86	21	86	1.76 ±0.17	11*	81	284.31 ±12.34	9	5 ±2.86	32 ±9.61
2015	CUBA1TEO30 x NS1	Program	8.99 ±0.86	22	85	2.18 ±0.17	34	101	269.28 ±12.34	18	3.33 ±2.86	32 ±9.61
2015	DK68-04	Check	8.92 ±0.88	23		1.65 ±0.17	5*		244.92 ±12.34	39	2 ±2.86	0 ±9.61
2015	NC_CK2	Check	8.92 ±1.04	24					244.62 ±13.47	40		
2015	ANTIGO2 x SS1	Program	8.78 ±0.86	25	83	2.12 ±0.17	31	98	262.26 ±12.34	31	9.67 ±2.86	27.33 ±9.61
2015	CUBA1TEO33 x NS1	Program	8.76 ±0.86	26	83	1.73 ±0.17	8*	80	255.91 ±12.34	35	0.67 ±2.86	27.67 ±9.61
2015	GTA1R TP Yellow E-1 X B73	Program	8.7 ±0.86	27	83	1.98 ±0.17	23*	91	264.12 ±12.34	26	1.67 ±2.86	4.67 ±9.61
2015	LH195 X GT1318	Program	8.54 ±0.86	28	81	1.91 ±0.17	17*	88	255.94 ±12.34	34	1.67 ±2.86	25.67 ±9.61
2015	LH210 X GT1214	Program	8.39 ±0.88	29	80	1.88 ±0.18	15*	87	245.17 ±12.34	37	9 ±2.86	1.33 ±9.61
2015	GTA1R TP Yellow E-1 X Mo17	Program	8.01 ±0.87	30	76	2.1 ±0.17	28	97	252.65 ±12.34	36	7 ±2.86	47.33 ±9.61
2015	CUBATEO90 x NS1	Program	7.99 ±0.86	31	76	1.96 ±0.17	21*	90	230.02 ±12.34	42	3.33 ±2.86	1.33 ±9.61
2015	Mp13:9031 x Mp13:9032	Program	7.92 ±0.86	32	75	1.65 ±0.17	4*	76	299.19 ±12.34	5	6 ±2.86	60.67 ±9.61
2015	8waf BULK2	Program	7.87 ±0.86	33	75	1.84 ±0.17	14*	85	297.92 ±12.34	6	2 ±2.86	35.67 ±9.61
2015	8waf BULK1	Program	7.42 ±0.86	34	70	2.04 ±0.17	25*	94	279.14 ±12.34	11	8.67 ±2.86	54 ±9.61
2015	Mp13:9013 x Mp13:9014	Program	7.38 ±0.86	35	70	1.81 ±0.17	12*	84	303.01 ±12.34	4	6 ±2.86	64 ±9.61
2015	Oh43 x FAW 1430	Program	7.07 ±0.86	36	67	2.03 ±0.17	24*	94	264.6 ±12.34	24	22.67 ±2.86	27.33 ±9.61
2015	8waf BULK3	Program	6.92 ±0.86	37	66	1.74 ±0.17	9*	81	297.44 ±12.34	7	10.67 ±2.86	65 ±9.61
2015	FAW 1430 x NC358	Program	6.82 ±0.86	38	65	1.94 ±0.17	19*	90	262.36 ±12.34	30	8 ±2.86	75 ±9.61
2015	Mp13:9037 x Mp13:9038	Program	6.63 ±0.88	39	63	1.59 ±0.17	2*	74	315.27 ±12.34	2	5.67 ±2.86	59.67 ±9.61
2015	Mp13:9027 x Mp13:9028	Program	6.42 ±0.86	40	61	1.7 ±0.17	7*	79	305.13 ±12.34	3	3.67 ±2.86	77.67 ±9.61
2015	Mp13:9021 x Mp13:9022	Program	5.63 ±0.86	41	53	1.56 ±0.17	1*	72	316.37 ±12.34	1	2.67 ±2.86	62.67 ±9.61
2015	CUBA1TEO21 x NS1	Program	5.21 ±0.87	42	49	2.49 ±0.17	39	115	230.87 ±12.34	41	3 ±2.86	41 ±9.61

‡ **Days to silking BLUPs ranged from 51 - 78 days from 2011-2015. Details provided upon request**

APPENDIX B

RNA-SEQ



Appendix 8. Normalized read counts of α zein genes at different stages of maturity and under different treatments. A - blister, B - Milk, E - Dough, F - Dent D - side needle inoculation, K - silk channel inoculation, N - no inoculation

Appendix 9. Annotation of fungal genes expressed in maize kernels at different stages of maturity					
Identified Fungal Genes	**Blister**	**Milk**	**Dough**	**Dent**	**Annotation**
ATP synthase					ATP synthesis
fucose-specific lectin FleA					carbohydrate binding/human disease
1,3-beta-glucanosyltransferase					carbohydrate metabolism
endoglucanse					carbohydrate metabolism
enolase/allergen Asp F 22					carbohydrate metabolism
fructose-bisphosphate aldolase					carbohydrate metabolism
glyceraldehyde 3-phos dehydrogenase					carbohyrate metabolism
plasma membrane H+-ATPase					cell metabolism
thiamine biosynthesis protein					cell metabolism
thiozole biosynthesis enzyme					cell metabolism
alcohol dehydrogenase					cell metabolism / detox of alcohol
aldehyde dehydrogenase					cell metabolism / detox of aldehyde
cytochrome c oxidase					cellular respiration
extracellular 3-ketosteroid 1-dehydrogenase					cellular respiration
mitochondrial F1 ATPase subunit alpha					cellular respiration
pyruvate decarboxylase					cellular respiration (anaerobic)
FKBP-type peptidyl prolyl isomerase					chaperone activity
histone H3					DNA replication, repair, transcription
histone H4					DNA replication, repair, transcription
histone H4.1					DNA replication, repair, transcription
woronin body major protein precursor					fungal hyphae protection
Bax Inhibitor family protein					ion transport regulation
allergenic cerat0-platanin					pathogenesis / elicitor of host response
allergen Asp F3					pathogenesis / allergenic response
pectinesterase precursor					pathogenesis / cell wall degradation
Appendix 9. Continued					
polyubiquitin UbiD/Ubi4					protein degradation
Glutamate/Leucine/Phenylalanine/Valine dehydrogenase					protein metabolism
glutamine synthetase					protein metabolism
peptidyl-prolyl cis-trans isomerase					protein metabolism/chaperone
Ribosomal Protein 40S					protein synthesis
Ribosomal Protein 60S					protein synthesis
transl elong factor eEF-3					protein synthesis
Appendix 9. Continued					
transl elong factor EF-1					protein synthesis
transl init factor eIF-5A					protein synthesis

Appendix 9 Continued.

translation initiation factor 4					protein synthesis
nucleoside diphosphate kinase					signal transduction
phosphoglycerate kinase PgkA					signal transduction
Aha1 domain family					stress response
CipC-like antibiotic stress responsive protein					stress response
flavohemoprotein					stress response
Hsp12					stress response
HSP30/HSP42					stress response
Hsp30-like					stress response
Hsp70					stress response
Hsp90/Hsp1					stress response
Mod-E/Hsp90/Hsp1					stress response
superoxide dismutase					stress response
actin Act1					structural
GPI-anchored cell wall org protein					structural
anchored serine-rich protein					structural
Appendix 9. Continued					
bZIP transcription factor CpcA					transcription regulation
BYS1 domain protein					unknown function
extracell serine-threonine rich protein					unknown function
high expression lethality protein Hel10					unknown function
Uncharacterized					uncharacterized
Number of Unique Transcripts Detected (No. reads ≥ 10)	70	7	25	100	
Key to chart: Med blue: side needle only, Red: side needle and silk channel, Light blue: side needle, silk channel and noninoculated					